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CONTENTS

REVIEW ARTICLE

ORIGINAL ARTICLES

Review Article

TREATMENT MODALITIES OF HYDATID CYST OF THE LIVER

Hikmat A.R. Hatam *FRCS*

Treatment options for hydatid cyst are[1]:-
1. Drug therapy (Medical).
2. Percutanous drainage.
3. Endoscopic therapy.
4. Laparoscopic surgery.
5. Surgery.

1- Drug therapy (Medical)

Two benzimidazoles (mebendazole and albendazole) and praziquantal (an isoquinolone derivalive) have scolicidal activity and have been used in over 1000 well-documented patients[1,2].
Mebendazole is poorly absorbed, and scolicidal drug levels are achieved with a daily dose of 40-50 mg / kg administered orally in divided doses[3].
Albendazole is better absorbed, and the usual daily dose is 10-15 mg / kg. Drug dosing with a fat-rich meal improve intestinal absorption. Cyclic treatment (three, 1-month covers with intervals of 14 days) has been widely used. Recent data show that uninterrupted drug therapy for 3-6 months has better efficacy with no increase in adverse effect[1,4].
Praziquantal is administered at a dose of 40 mg/kg concomitantly with benzimidasoles shown to increase the scolicidal activity of imidazoles. Chemo-therapy achieves a cyst disappearance of 30%, partial response in another 30% and no response in 40%. It is effective in small cysts (< 4 cm dia.), cyst with thin wall and younger patients[1,5,6].
Medical treatment is indicated in patients who are risks for surgery, in patients with multiple peritoneal cysts, to prevent secondary echinococosis, after spillage during surgery or after trauma and as a concomitant therapy with percutanous drainage. Imidazoles are hepatotoxic, can cause neutropenia, thrombocyto,penia, alopecia and are potentially teratoeaenic[1-4].

2- Percutanous Drainage

It is also known as PAIR (Puncture, Aspiration, installation of hypertonic saline scolicidal agent and Reaspiration). The procedure is minimally invasive, cost-effective, involved reduced hospital stay and has less morbidity and mortality than surgery. It is the treatment of choice in patients who refuse surgery or have significant co-morbid diseases.
The procedure is highly effective in cysts with few large daughter cysts, cysts with diameter. Over 5 cm and multiple cysts in different liver segments, cyst relapsing after surgery or failed to regress following chemotherapy.
The procedure can not be done in small volume cysts, cysts with honey comt appearance (multiple small daughter cysts), cysts with dominant solid component and those with infection or communicating with bile ducts.
The procedure is associated with possible complication of liver puncture (bleeding, bile peritonitis), anaphylaxis, allergic reactions and biliary communication. Inadverent installation of sclerosing agent into a cyst with biliary communication can cause sclerosing cholangitis[1,7].

3- Endoscopic therapy

It is used in a small number of patients with hepatic cysts which have ruptured into bile ducts. At ERCP, endoscopic sphincterotomy is performed; laminated membrance and daughter cysts are extracted, and a nasobiliary drain in

Head of the Dept. Surgery, College of Medicine, Al-Nahrain University

placed in the bile duct. Also used to manage persistant bile fistula after surgical cystectomy[1,8].

4- Laparoscopic surgery

Laparoscopic removal of hepatic cysts has been reported but this modality is not evaluated in controlled trains[1,8].

5- Surgery

At present, surgery offers the only consistently effective treatment for living abdominal hydatid cyst[1,8,9].

The objectives of surgery are:

A- Total removal of all parasitic elements.
B- Avoidance of spillage of cyst contents.
C- Management of residual pericyst cavity.

A. Total removal of all parasitic elements

There are basically four ways to manage hydatid cyst of the liver.

I. Hepatectomy

Used usually for multiple hydatid cysts confined to anatomically resectable segment(s) of the liver. Lobectomy is an acceptable method for surgical management of intact cyst(s) in the left lobe of young patient who do not reside in endemicarea[1,9].

II. Pericystectomy

Applied to small perdiculated cysts. Also called radical cystectomy where it involve resection in the normal liver tissue with the complications of being bloody and possibility of bile leak[1,8,9].

III. Enculeation

Delivery of the parasite intact by opening the potential space between the laminated membrance and the pericyst then management of the cavity[1,8,9].

IV. Evacuation of cysts

This is accomplished by opening the hydatid cyst and evacuation of the whole content, then dealing with the residual cavity[1,8,9].

B. Avoidance of spillage of cyst contents

This can be achieved by:
I. Careful surgical technique.
II. Simple packing of the operative field isolating it from the peritoneal cavity.
III. The use of hydatid cones; Spillage of hydatid fluid is now very effectively prevented by the use of hydatid cones, through which the operation can be performed. These are of two types[1]:

*Cryogenic cone

This adheres to the hydatid cyst surface by freezing and kill any spilling fluid after incision, this technique suffer from potential problems which are:-
a. Risk of damage to other structures suck as the bowel coming into contact inadvertently with freezing ring.
b. The necessity to excise that portion of cyst wall damage by the freezing[1].

*Suction cone

It is basically the same as cryo-cones but it adhere to the cyst by suction applied to the cone that effectively such any spilling fluid avoiding the potential problems of the cryo-cones[1].

C. *Management of Residual pericyst cavity*[1,8,9]

i. Primary closure:

In the case of a clean, simple cyst with smooth adventitia the cavity is filled with sterile normal saline and closed.

ii. Obliteration of the cavity

This is not recommended for large cyst owing to the difficulty of obliterating the cavity and the danger of including large bile ducts in the suture line.

iii. Drainage of the cavity

This way is preferred in case of infected hydatid cyst, large cavity remaining, suspicious of bile leak and uncertainty about complete removal.

iv. Omentoplasty

The results with this technique are very favorable. It reduces the hospital stay

while the incidence of biliary leaking is minimized from 25% to 2.5%. So it is considered the treatment of choice in hydatid of the liver.

v. Cystoenterostomy
Usually done in case of infected hydatid or cases of bile leak where the cyst is opened into a viscous either to the stomach or to the small intestine by forming a Roux-en-Y defunctiolizied loop.

Management of intrabiliary rupture
Almost always the cause of jaundice in association with hepatic hydatid cysts is rupture into the biliary ductal system, under these circumstances; an operative cholangiogram is most helpful for the diagnosis. Hopefully, when the operative cholangiogram is normal, the hydatid elements are so small that they will pass spontaneously if undetected at surgery. Firstly the liver cyst is dealt with in exactly the same manner as for unruptured cyst with parlicular care taken to identify and suture the biliary communications responsible for the hydatid elements entering the ductal system. The cavity should not be drained but should be closed after filling with normal saline to buffer the intrabiliary pressure. Then the CBD is explored and any hydatid elements are extracted. Endoscopic sphincterotomy, preliminary to surgery, is particularly useful in the poor risk patient whose main presentation is with obstructive jaundice. When jaundice is relieved, the surgery can proceed to definitive procedure to remove the liver hydatid. It is found useful at the completion of bile duct exploration to perform choledochoscopy[1,9].
These surgical techniques carefully followed have almost eliminated postoperative biliary fistula. Very occasionally a large biliary communication resist closure due to calcification of the wall, and in such cases a technique that is omentorplasty is useful. In some very persistent cases, when the residual cavity had been formally drained, a persistent biliary fistula that dose not close within a few months need re-operation. In the absence of CBD obstruction, the most suitable form of secondary surgical intervention is internal drainage of this fistula into a defunctionalised segment of jejunum[1,8,9].

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Original Articles

HYDROCORTISONE INDUCED DIFFERENTIATION OF EMBRYONIC STEM CELLS *IN VITRO* TO NEURONAL- LIKE CELLS

Mahmoud H. Hammash[1] *MBChB Ph.D.*, **Intissar N. Waheed**[2] *B.Sc. Ph.D.*

Abstract

Background: Embryonic stem (ES) cells, derived from the inner cell mass of murine blastocysts, can be maintained in a totipotent state in vitro. In appropriate conditions ES cells have been shown to differentiate in vitro into various derivatives of all three primary germ layer.

Objectives: To report the derivation of neuronal-like cells from murine (mouse) ES cells that remain undifferentiated in continuous passage for (3 months).

Methods: Mouse ES cells obtained from cultured blastocyst were subjected to an optimized in vitro differentiation protocol to obtain aggregate and embryoid bodies (EBs). These aggregates and EBs after passage and form monolayer were treated with Hydrocortisone for a precise period of time.

Results: Treatment with Hydrocortisone resulted in the subsequent appearance of neuronal- like cells in the culture.

Conclusion: Hydrocortisone, here play an important role to direct differentiation of ES cells to neuronal pathway.

Key words: Mouse embryonic stem cell, Neuronal differentiation, Hydrocortisone.

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Introduction

Embryonic stem (ES) cells isolated from murine pre-implantation embryos can be maintained as stable cell lines in culture and following the appropriate stimuli in vitro or in vivo will differentiate into variety of cell type[1-3]. Thus ES cells can be used to study embryonic growth control and differentiation in culture[4].

In vitro, ES cells have been shown to differentiate spontaneously into various lineages such as extra embryonic yolk sac, cardiac, haematopoietic[4], and skeletal muscle[5] and epithelial aggregates[6]. Differentiation events similar to those in vivo can be obtained in cell culture using ES cells[7]. The abilities to direct pluripotent ES cells into specific pathways and then to support the viability and maturation of individually differentiated phenotypes in vitro are currently limited and the approaches rather unsophisticated. The direct derivation of ES cells is then vital to the ultimate use of such cells in the development of new therapies. The use of ES cells to generate tissue that could be used in treatment of neurological diseases is a major focus of researches, particularly on spinal cord injury, stroke, multiple sclerosis and Parkinson's diseases of which the concept of replacing damaged or dysfunctional cells in the brain or spinal cord is a practical goal[8].

The principle method used to trigger differentiation of ES cells into defined cell types is first, cell aggregation in suspension culture. This technique, leads to formation of multidifferentiated structures called embryoid bodies (EBs). In these structures, the developmental programs of inner cell mass (ICM) / epiblast cells are reactivated in the ES cells. Cellular differentiation proceeds on a schedule similar to that in the embryo

[1]Dept. Human Anatomy [2]Research Center (College of Medicine, Al-Nahrain University).
Address correspondence to Dr. Intissar N. Waheed, P.O. box 70069, e-mail:- intissar1960@yahoo.com
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but in the absence of proper axial organization of elaboration of a body plan[3,4]. Each EB develops multiple cell types, and further differentiation is elaborated on subsequent attachment and outgrowth. Second, adding growth factors to the culture medium of these aggregate and EBs[9].

According to Benninnger et al[10], they were demonstrating that's when ES cells culture medium was supplemented with the following additives in various combinations: Hydrocortisone, retinoic acid, insulin, transferrine, selenite, biotin and growth factors, their differentiation was directed to the neuronal pathway.

Here, we show that applying this knowledge and in vitro, tested whether addition of hydrocortisone only with culture medium to the monolayer of the aggregates and EBs outgrowth directs their differentiation into neural pathway?

Hydrocortisone (cortisol) is the main glucocorticoid hormone secreted by the adrenal cortex. One of their principal pharmacological actions was their effect on tissue repair (the heart, kidney and CNS)[11]. The mechanisms of action of the glucocorticoids, after entering cells, bind to specific receptors in the cytoplasm. These receptors, which have a high affinity for glucocorticoids are found in virtually all tissue, at about 3000 to 10000 per cell. The number varies in different tissues. After interaction with steroid, the receptor become "activated", i.e. undergoes a conformational change, which exposes a DNA- binding domain. The steroid- receptor complexes form dimers (pairs), then move to the nucleus and bind to steroid-response elements in the DNA. The effect is either to repress (prevent transcription of) or induce (i.e. initiate transcription of) particular genes[12].

The present study deals with the establishment and maintenance in culture, of pluripotent mouse ES cells and using these cells to study differentiation events in neurogenesis. The latter was essential to characterize in more detail the ES cell-derived neuron-like cells and to determine conditions of culture that are favorable to direct the differentiation of ES cells to the neuronal pathway.

Materials & Methods

Albino mice of (strain: Balb C) were obtained from the (Animal Breeding House of Medical Research Center / College of Medicine)**,** were selected and set up for breeding. Females were mated with males and blastocysts were obtained by flushing the uterus at day 3.5 after natural mating-had taken place[3,13].

Establishment of ES cells culture:

Three embryos (blastocysts) were plated onto a feeder layer of mitomycin-C treated embryonic fibroblast[1,3] in Minimum Essential Medium modified (MEM) Eagle (Sigma) supplemented with 20% New Born Bovine Serum (NBBS) (Sigma) in 4-well plates (Nunc).

Most embryos hatched and attached to the feeders within 24 hours of plating. The ICMs were left to grow for 6 more days, then, they were mechanically disaggregated by using drawn-out pasture pipettes and returned and cultured in the same plate wells[1,3,13].

Seven days later, the cells were transferred into new wells containing feeder layer, either by trypsinizing or by mechanically disaggregating the undifferentiated colonies. The cells were passaged for several times till 3 months[3,13].

ES cells culture and differentiation of embryoid bodies:

For differentiation, after 3 months of ES cells culture, cells were trypsinized into single cell suspension, then plated and cultivated in the absence of (feeder layer and substrate coated dish), in non adhesive bacteriological petridish in the same medium as that used for initial ES cell culture. The resulting, formation of aggregates and EBs[3,9,10].

To initiate differentiation, the aggregate and EBs were transferred into substrate (0.1% gelatin) coated plate well and maintained for 3 days to form monolayer

in the same medium as that used for initial ES cell culture[3,14,15].

Then, medium was changed with a defined medium that favors the survival of ES cell-derived neural precursors[3] with some modification and as described in the following protocol:

Monolayer were sequentially propagated through media containing (a) 20% NBBS and 10 µg /ml hydrocortisone* for two weeks. Medium was changed every two days. Then (b) 20% NBBS and 20µg /ml hydrocortisone for two days.

The plates were inspected daily for differentiation.

*Hydrocortisone used in this study (as cortisolone succinate sodium 0.13372 gm). /Hemol.

Results

Establishment and maintenance of the ES cells:

After approximately seven days of culturing blastocysts on a mitomycin-C treated embryonic fibroblast feeder layer, clumps of ICM cells from the blastocysts (Figures 1A, B & C), were pick out, mechanically dissociated by pipetting and returned to the same feeder layer. Within two days of the culture, these fragments attached and proliferate to form large primary ES cells colonies (Figure 2A), this stage marked as passage No-1. Each putative ES cells colony was dissociated then cultured (this stage marked as passage No-2). As long as the cells were maintained on a dense layer of feeder cells and replated every week on a new feeder layer, differentiation was inhibited. This was determined morphologically. When carefully maintained in culture in the undifferentiated state as described above, newly formed colonies of pluripotential ES cells are identified in culture after 5-6 days from the starting passage No-2.

The cells in the early passages were packed tightly together in small nests in which it is difficult to discern the individual component cells (Figure 2B), and several small and large colonies of ES cells are formed in the advanced passages and these colonies are spread and flatten, the constituent cells also adhere tightly to one another, this growth patter is characteristic of stem cells.

ES cells can be kept in culture for 3 months to more time without any apparent lose in developmental potential. If the cells were grown on tissue culture plates without feeder layer, differentiating, substrate-attached cells began growing out from the undifferentiated cell clumps within 24 hr such as fibroblast, endoderm, trophoblast cells.

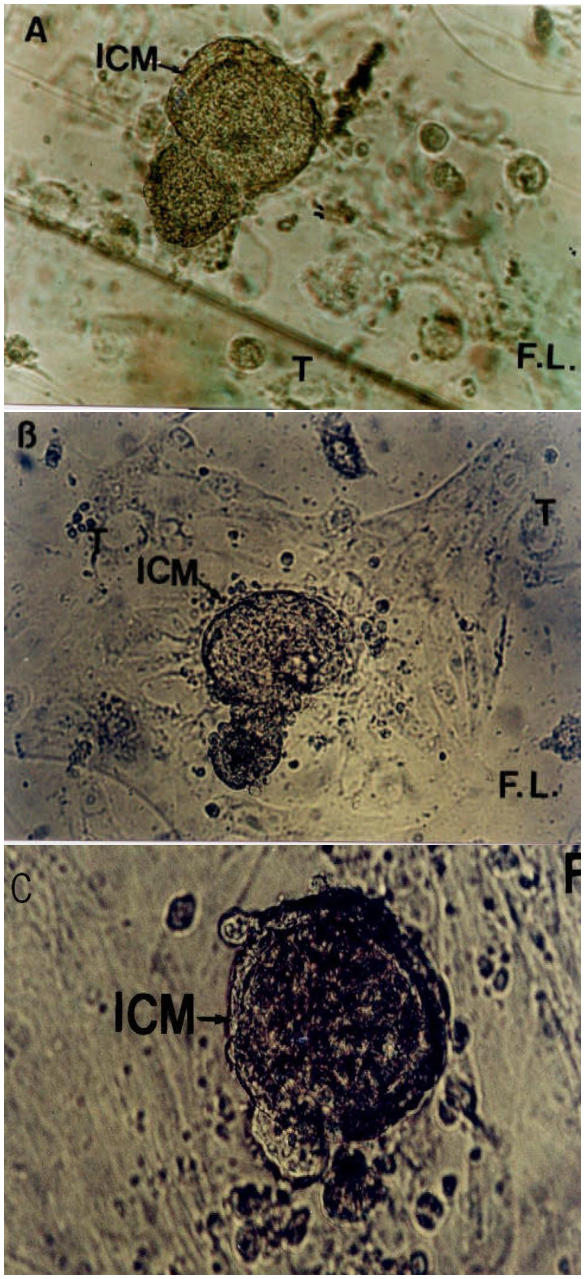
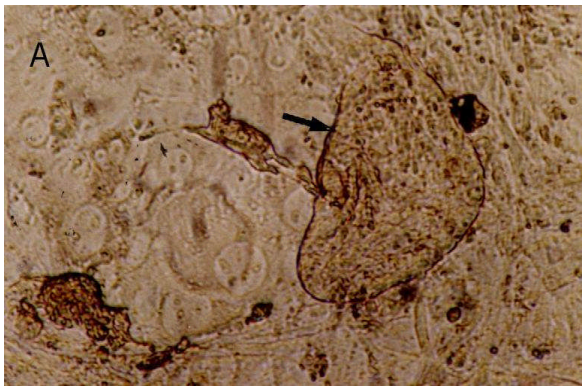


Figure 1: (A, B & C) Attachment and growth of a single blastocyst on top of the feeder layer (F.L.) by trophoblast cell (T) outgrowth. Inner cell mass (ICM) has formed amass of cells (→). (Living material). A: 4 days culture (x100.8). B: 6 days culture (x100.8). C: 7 days culture (x60)



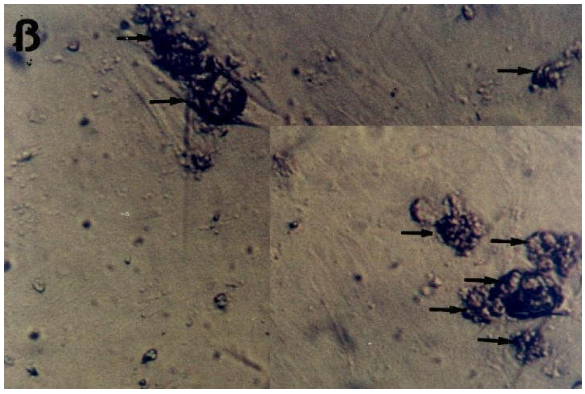


Figure 2: (A & B) Morphology of ES cells colonies (→). (Living material). A: Primary colony of ES cells (x63). B: Appeared as compacted stem cell colonies with intact borders (x160).

Pluripotential cells are typically small, have a high neucleous / cytoplasm a ratio and prominent nucleoli (Figure 3).

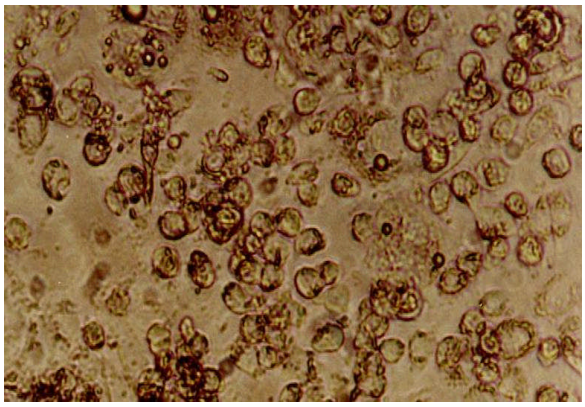
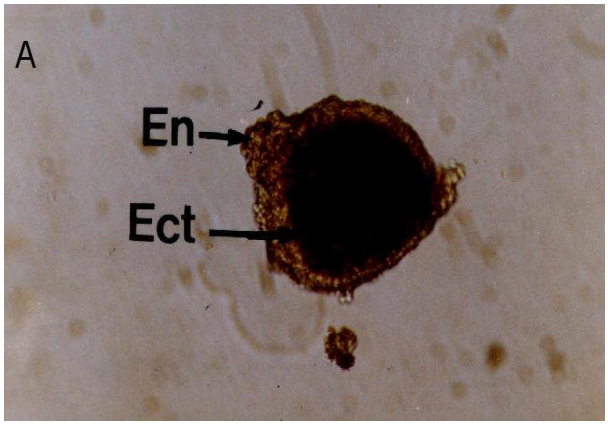


Figure 3: Pluripotential embryonic stem cells, which have a high ratio of nucleus to the cytoplasm (Living material) (x160).

The ES cells culture should be passaged when the stem cells have reached an approximately 80% confluence. The ES cell culture should be maintained a relatively high density to ensure maximal growth rate in order to minimize spontaneous differentiation.

Developmental of ES cells:

Differentiation capabilities and developmental potential of ES cells at different passages were tested in vitro by differentiation the cells into EBs. That's when ES cells were passaged in suspension without feeder cells and substrate attached cell, they formed aggregates and EBs with an outer layer of endoderm and the inner cells the condensed layer of columnar ectoderm-like cells (Figure 4A). Which when kept more in suspension became cystic EBs (Figure 4B).



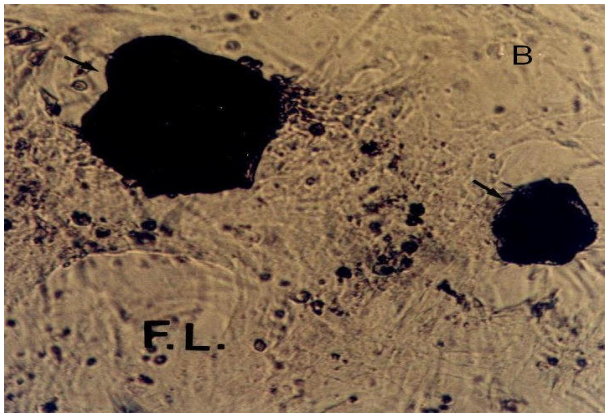


Figure 4: A: Embryoid body, derived from ES cells culture under differentiation condition. (Living material). The outer layer of endoderm (En). Inner cells (condense layer) embryonic ectoderm like cells (Ect).(x40). B: Cystic EBs, derived from ES cells suspension culture, attached to the feeder layer (F.L.) (Living material). (x40).

Differentiation of ES cells into neuronal-like cells:

For directed differentiation, we were investigated the capacity of ES cells to undergo neural differentiation following exposure to hydrocortisone. The protocol is described in materials and methods appeared more suitable to promote the differentiation of ES cells to the neuronal pathway.

After a period of 2-5 days in suspension culture, ES cells formed aggregates and EBs. The latter's were plated to form monolayer and were maintained in differentiation medium containing hydrocortisone and within the time of the culture, ES cells appeared to differentiate to neuronal-like cells (Figure 5A) and at the end of experiment amonolayer of differentiated ES cells of neuronal-like cells were observed in the plate well (Figure 5B).

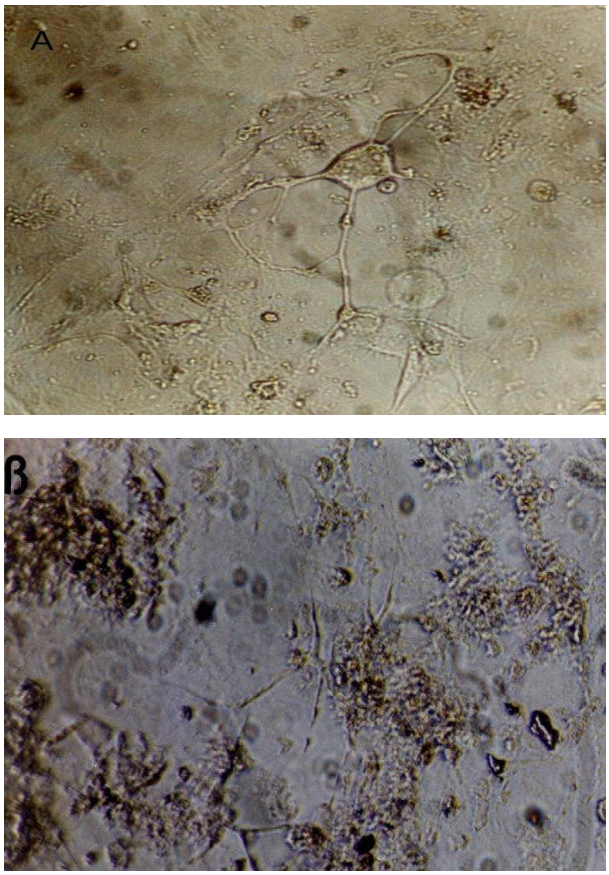


Figure 5: A & B: Morphology of in vitro differentiation of ES cells to the neuronal-like cells. (Living material). A: x100.8 B: Group of differentiated ES cells to neuronal-like cells growing in monolayer (x40).

Discussion

Isolation and maintenance of the ES cells in the undifferentiated state in vitro requires the presence of a feeder layer of growth arrested embryonic fibroblast[1,3,4].

From the result of the present study and other studies[1,3] appeared that's mitotically inactivated feeder layer (embryonic fibroblast cells) is sufficient for isolation and maintained a pluripotent state of ES cells cultured in vitro and that feeder cells thought to have generalized effects such as media detoxification, in addition to more specific effects in promoting cell attachment and viability and preventing ES cell differentiation[2,3].

Pluripotency, that is the ability to give rise to differentiated cell types that are derived from all three primary germ layers of the embryo (endoderm, mesoderm and ectoderm), were tested in this study, that's when ES cells were removed from feeder layers and transferred to suspension culture, ES cells begin to differentiate into multicellular aggregates of differentiated and undifferentiated cells, forming EBs which resemble early post-implantation embryos and these structures may yield important new information on early inductive events in mammalian development[3,16,17].

ES cells capture the imagination because they are immortal and because they have an almost unlimited developmental potential. After long-time of growth in culture dishes, these cells maintained the ability to form cells ranging from nerve to potentially any cell type that makes up the body[18,19].

Stem cells have the ability to choose between prolonged self-renewal and differentiation. This fate choice is regulated by intrinsic signals and the external microenvironment, the elements of which are being rapidly elucidated. These cells will need to be differentiated or otherwise modified before they can be used clinically[20,21].

The differentiation of ES cells into specialized population need to change the growth conditions of the ES cells in specific ways[9,10]. In the present study we

describe the capacity of ES cells to undergo neurogenesis at a high rate in vitro after treatment of developing ES cell derived- aggregates and with hydrocortisone.

Glucocorticoid effects involve interaction between the steroids and intracellular receptors that belong to the superfamily of receptors that control gene transcription. This superfamily also includes the receptors for mineralocorticoids, the sex steroids, thyroid hormones, vitamin D3 and retinoic acid (RA). There are believed to be 10-100 steroid-responsive genes in each cell[11,12]. RA for example, is a critical regulatory signal molecule in developmental process. In vitro, it has been reported that RA influences in a time and concentration-dependent manner the efficiency and pattern of differential of ES cells[15]. Here, we show that applying this knowledge to the hydrocortisone that belong the same superfamily of the RA as described above to induced differentiation of ES cells in vitro to obtain neural precursors.

From the result of the present study, hydrocortisone plays an important role in in vitro differentiating pattern of ES cells to the neuronal-like cells. To explain the possible mechanism of hydrocortisone on the differentiation of ES cells was very difficult and its need further studies to explain how the directed differentiation occurs, how or when gene expression is changed, or what signal-transduction systems are triggered, or what cell-cell interactions must occur to convert undifferentiated ES cells into precursors cells.

To our Knowledge this study is the first report that hydrocortisone induced differentiation of ES cells to the neuronal-like cells.

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IN VIVO OXYTOCIN TREATMENT INDUCES ACCIDENTAL THYMIC INVOLUTION IN MICE

Linda S. F. Ali[1] *Ph.D.*, **Haider S. Abid** *M.Sc.*

Abstract

Background: Oxytocin (OT) was found to be produced by thymic epithelial cells and considered as the self antigen for the neurohypophysial hormone family in the thymus. It was postulated that this neurohypophysial hormone has a dual role both in the positive and negative selection processes of thymocytes. Attempts are recently being made to elucidate the effects of high levels of OT on the immune function of animal models.

Objective: To investigate the effects of OT on the histology and physiology of the thymus gland in vivo.

Methods: Twenty albino male mice with 4 weeks of age were injected with OT(with doses of 0.2ml\ day of 0.5, 1.0 or 2.0 IU; for 10 days).The histological study was performed using the routine hematoxylin-eosin (H&E) staining technique in addition to the periodic acid- Schiff´s (PAS) reaction. Thymic parameters were measured using ocular and stage micrometers.

Results: Thymus cellularity, architecture, and weight were markedly affected by the treatment with each of the three concentrations of OT. OT caused increases in cell death, necrosis, phagocytic activity, and cyst formation in the thymic tissue as well as signs of hemorrhage and engorgement within the capillaries of the thymus. Reductions in thymus weight and in the width (thickness) of the thymic cortex as well as an increase in the diameter of Hassall's bodies were also observed.

Conclusion: OT, with the concentrations used in the present study, is able to induce an accidental involution in the thymus. The results also provide an in vivo support to the role of OT in T cell negative selection within the thymus.

Keywords: Oxytocin, thymus, mice.

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Introduction

A reciprocal interaction between the endocrine and immune system is now largely established[1], especially at the level of thymus where such interaction is obvious since thymic hormones can modulate the production of classic peptide hormones[2] and, by contrast, the hormones of adenohypophysis[3,4], thyroid[5,6], and adrenal[7] glands can modulate thymic physiology. Also, thymic epithelial cells (TEC) can produce and express receptors for hormones such as GH, PRL, ACTH, TSH, T3, FSH, LH, arg-VP, and OT[8]. Basically, the model of thymic T-cell education to neuroendocrine self was established by the identification in thymic nurse cells (TNC) of OT as the self antigen of the neurohypophyseal (NHP) family[9]. OT is locally synthesized by TEC[10] and TNC[11], while receptors for OT are present on rat thymocytes[12] and murine pre-T-cells[13]. These findings and the previously reported mitogenic[14] and immunomodulatory[15] properties of OT upon thymocytes and TEC in vitro strongly support the existence of a neuroendocrine-thymic axis and an active role for thymic oxytocin in T-cell positive and negative selection. Given the above information, the present study was designed to reveal more about the proposed role for OT on thymic physiology and T-cell differentiation in vivo using a mouse model and employing the histopathological criteria in addition to the standard thymic parameters.

Materials & Methods

Animal Raising: Male and female albino Swiss mice (Mus musculus) were originally purchased from Al-Razi center for research and medical diagnostic

[1]Dept. Medical Biology, College Of Medicine, Al-Nahrain University. [2]Dept. Biology, College of Science for Women, University of Baghdad. Address of Correspondance to Dr. Linda S. F. Ali, e-mail: lindaali20002000@yahoo.com

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production and they were raised, maintained, and allowed to mate in the animal house, Department of Biology, College of Education Ibn Al-Haitham. The diet, supplied from Eba Center for Agricultural Research, was fed ad libitum while conditions of 12h light: 12h dark cycle and temperature ranging 20-26^{o}C were ensured[16]. Mothers and their litters were housed in plastic maternity cages and the males were then weaned at 3 weeks of age and isolated in special cages for the purposes of the experiments.

Animals and treatment: Twenty albino male Swiss mice with 4 weeks of age were used in the present study and divided into two groups. Animals of the control group (n=5) were injected intra-peritoneally (IP) with 0.2ml of 0.9% physiological saline/ day for 10 days. Animals of OT- treated group were injected with 0.2 ml/day of 0.5IU (n=5), 1IU (n=5) or 2 IU (n=5) of OT for 10 days as well[17].

Collection of Samples: The animals were weighed first and then anesthetized with chloroform. Thymuses were removed and transferred to a sterilized Petri dish containing phosphate buffered saline (PBS) solution (pH 7.2). The glands were weighed and used in the histological study.

Preparation of Microscopic Slides: Thymuses were fixed in Bouin's solution for 14 hours and then preserved in 70% ethanol. After several washings with 70% ethanol, the glands were dehydrated with ethanol and then embedded with paraffin wax. Thin serial section (5 μm) were prepared and placed on glass slides[18].

Hematoxylin & Eosin (H&E) Stain: Sections were deparaffinized with xylen and then hydrated to water.Using Harris hematoxylin, sections were stained for 15 min, washed in running tap water for 30 sec., and then rinsed in DW. Sections were stained with eosin for 30 sec. and rinsed in DW.Following dehydration, sections were cleared with xylen and mounted with D.P.X[18].

Periodic Acid-Schiff's (PAS) Reaction: Sections were deparaffinized with xylene and then hydrated to DW.Oxidation of sections was performed using 1% periodic acid for 10 min. Sections were rinsed several times in DW for 5 min. After immersion in Schiff's reagent for 10 min., sections were washed with running tap water for 10 min to ensure the color formation. Counter staining was performed with Harris hematoxylin for 15 sec.Sections were washed with running tap water for 15 min., and then dehydrated, cleared with xylene, and mounted with D.P.X.[19].

Thymic Parameters: Thymys weight / body weight ratio (Th.Wt./B.Wt) was calculated after weighing thymuses using a digital balance (Sartorius Analytic). Cortex width / medulla width ratio (C.W./M.W.)[20] and the diameter of Hassall's bodies (HPs) for each treated group were determined using the ocular lens and then compared to those from the control groups.

Statistical Analysis: The data obtained were statistically processed using the analysis of variance (ANOVA) test for a factorial experiment[21] with the statistical significance was accepted for $P \leq 0.05$. The results were presented as a mean and standard error (SE). When the initial ANOVA indicated that it was appropriate, the effects of specific in vivo treatments were determined within groups separately and individual mean comparisons were made using the least significant difference (LSD) test.

Results

The following results were obtained after injecting the mice (4 weeks old) intraperitoneally with 0.2ml/day of the hormone OT for 10 days at a fixed time. The injection regimen included three

doses of the hormone (0.5, 1.0, or 2.0 IU) for each subgroup.

Thymus Weight/Body Weight Ratio (Th.Wt./B.Wt.): There were highly significant decreases in Th.Wt./B.Wt. after the treatment with each of the three doses of OT, and the highest decrease was observed in the treatment with 1 IU of OT (2.381±0.137, P< 0.0001), as compared to the control group (3.994±0.210) (Table1).

Table 1: The effect of 10 days treatment with different concentrations of OT on Th.Wt. / B.Wt.

Treatment 0.2 ml of	B.Wt. (gm)	Th. Wt.(gm)	Th. Wt/B.Wt.
0.9% saline a	25.925±0.747	0.103±0.006	3.994±0.210
0.5 IU, OT b	22.185±0.618	0.068±0.006	3.081±0.267
1 IU, OT c	23.897±1.004	0.057±0.005	2.381±0.137
2 IU, OT d	20.066±0.615	0.060±0.002	2.995±0.083

a, c : significant at p<0.0001; a, b ; a, d : significant at P<0.01

Cortex/Medulla width Ratio (C.W. /M.W.): The treatment with 1IU OT showed a significant (P<0.05) decrease in C. W. / M.W. (1.464±0.226) as compared to the control group (2.572±0.401), whereas the decrease was not significant (P<0.05) in the mice treated with 0.05 or 2IU of OT (Figure 1).

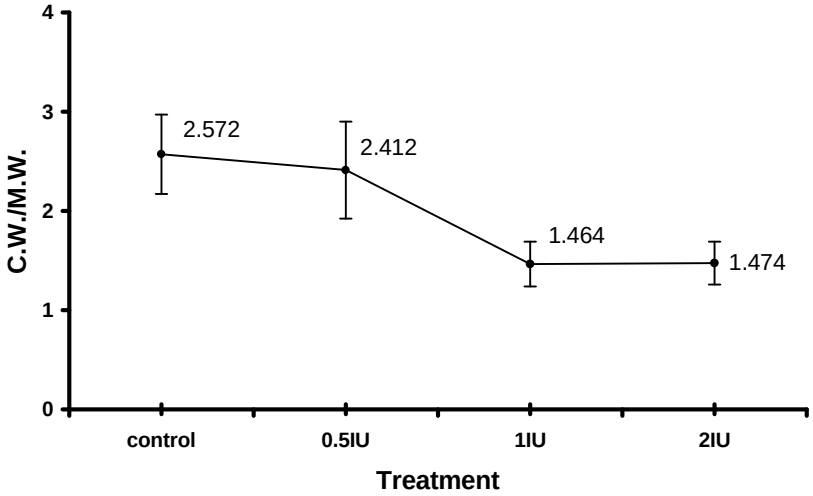

Figure 1: The effect of 10 days treatment with different concentrations of OT on thymic cortex width/medulla width ratio.

Diameter of Hassall's Bodies (HBs): The results showed a significant increase (P<0.05) in the diameter of HBs for each of the three OT treatments used in this study. The highest increase was recorded in the concentration of 1IU (13.790±0.155) µm as compared to the control group (10.889±0.202) µm (Figure2).

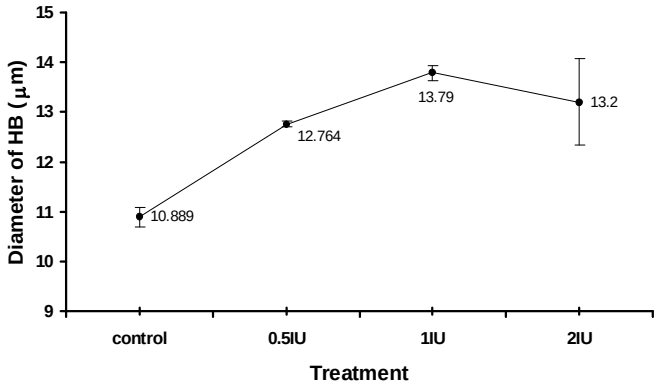

Figure 2: The effect of 10-days treatment with different concentrations of OT on the diameters of HBs (µm)

The Histological Study: Several histological changes were observed in the thymuses of mice after treatment with OT especially at the higher two concentrations (1 IU and 2 IU). These results were compared with the normal tissues from the control animals (Figures 3 and 4).

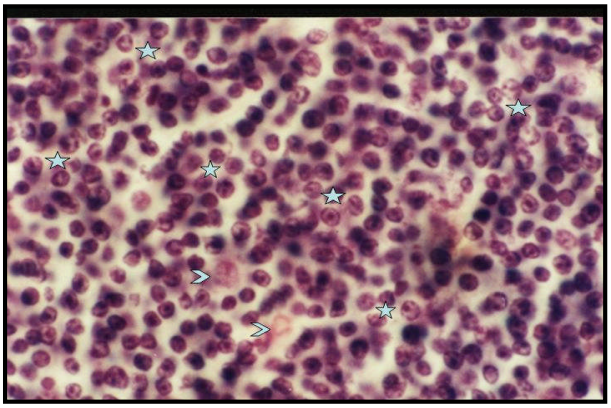

Figure 3: Cross section of the thymic cortex from control mice. The cortical cellularity is typically normal, with numerous thymocytes (asterisks) and few scattered epithelial cells (arrow heads). H &E, X 1000.

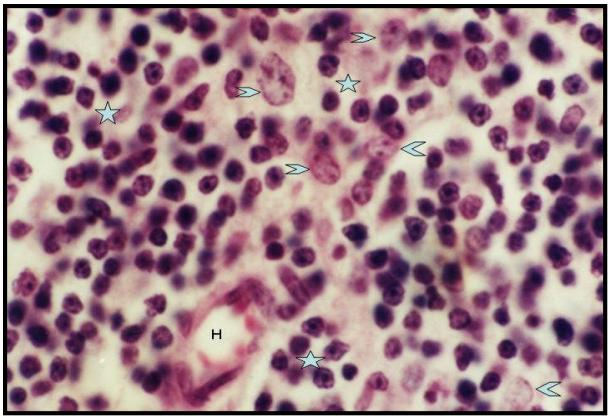

Figure 4: Cross section of the thymic medulla from control mice. The epithelial cells (arrow heads) are more evident with lower numbers of thymocytes (asterisks) comparing with the cortex. The normal Hassall's body (H) is also observed. H & E, X 1000.

Cellular destruction and death of cells were observed in thymic cells which were distributed in the various regions of cortex (Figure 5) and medulla (Figure 6).

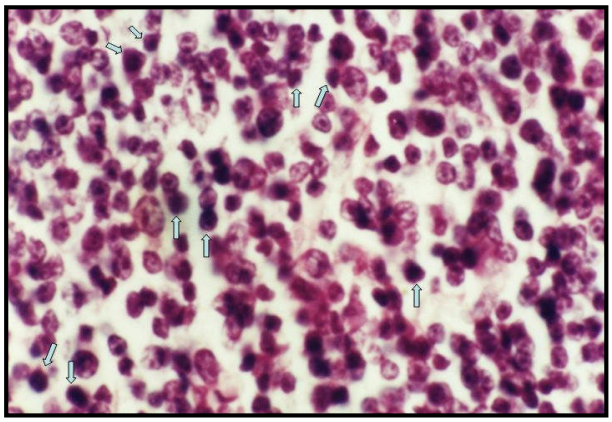

Figure 5: Cross section of the thymic cortex from mice treated with 1IU of OT. Numerous thymocytes with signs of destruction and cell death (Arrows) within necrotic cortical tissue. H & E, X1000.

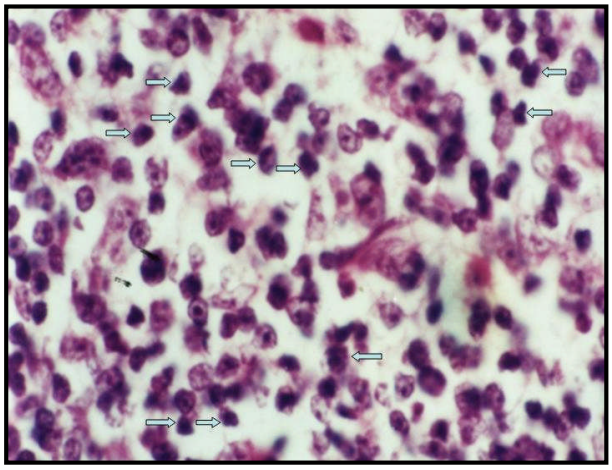

Figure 6: Cross section of the thymic medulla from mice treated with 2IU of OT. Destruction and death of thymic cells (Arrows) seen with marked necrosis of the medullar tissue. H & E, X 1000.

Also, high numbers of macrophages were exist throughout the gland, especially in the medulla (Figure 7).

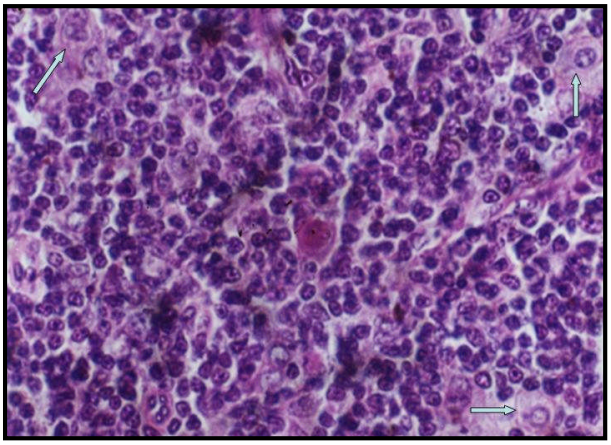

Figure 7: Cross section of the thymic medulla from mice treated with 2IU of OT. High numbers of macrophages (Arrows) invading the tissue and containing incorporated degenerating lymphocytes. H & E, X400.

They were large in size and containing a number of ingested thymic cells as well as cellular debris. At the tissue level, a necrotic appearance of the thymic tissue was obvious and widespread both in the cortex (Figure 5) and medulla (Figure 6). The forthcoming alterations were accompanied by cases of congestion within the capillaries in the cortex, with some of these vessels being expanded and disrupted resulting in an hemorrhagic appearance of the sorrounding tissue (Figures 8 & 9).

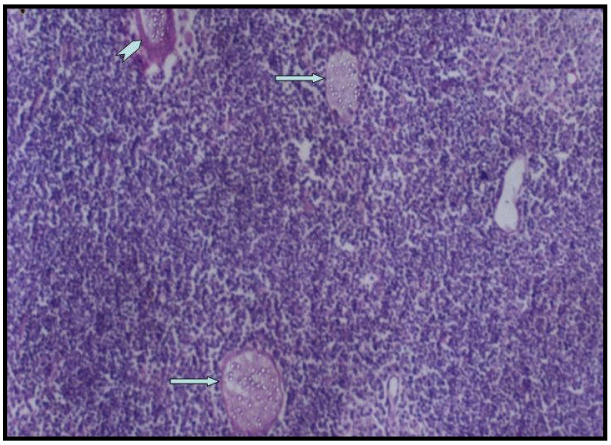

Figure 8: Cross section of the thymic medulla from mice treated with 2IU of OT. Notice the congestion of blood cells within the capillaries scattered in the cortex (Arrows). An obvious hemorrhage is also observed (arrow head). PAS X100.

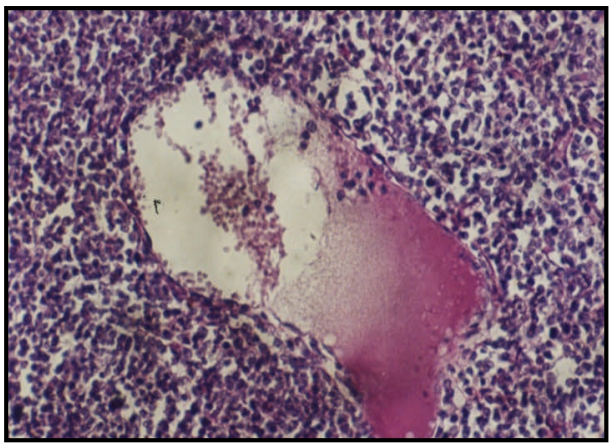

Figure 9: Cross section of the thymic cortex from mice treated with 2IU of OT. A large, expanded, and disrupted blood vessel with destruction of the thymic-blood barrier. PAS, X 400.

The medullary tissue of thymuses from OT-treated mice showed the existence of large-sized Hassall's bodies containing degenerating epithelial cells. In some cases, there was more than one Hassall's Corpuscle incorporated to each other and forming very large structures (Figure10).

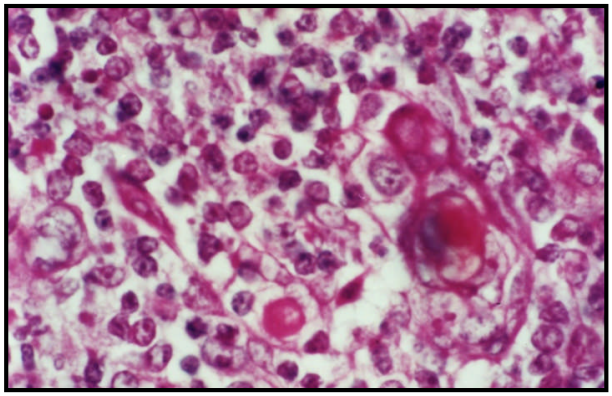

Figure 10: Cross section of the thymic medulla from mice treated with 1IU of OT. A large structure with ill- defined margins composed of two incorporated Hassall's bodies. H & E, X1000.

In addition, formation of thymic cysts was observed in some regions of the medulla, with these structures being lined with flattened epithelial cells and containing cellular debris (Figure11).

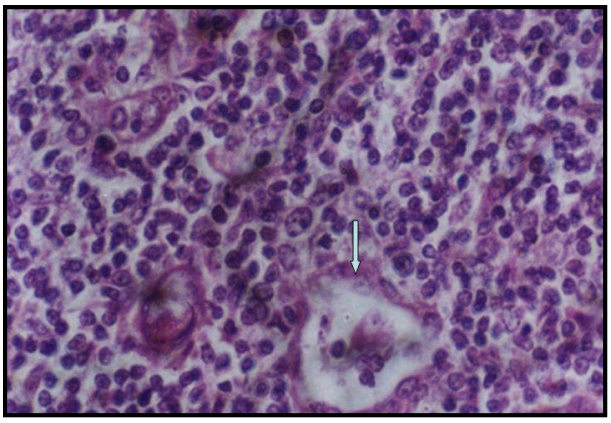

Figure 11: Cross section of the thymic medulla from mice treated with 0.5 1IU of OT. Notice the cyst formation (Arrow) lined by flattened TECs with cellular debris inside its lumen. H & E. X400.

Discussion

Thymys reaches its maximal weight between 4 and 5 Wks of age in mice[2]. Hence,the age of 4Wks has been selected in the present study to prevent the age-related involution of the gland .However,other types of involution exist and known to have initial triggering factors and a relatively "acute" mechanism where thymus has been shown to undergo a very rapid and sudden involution[22]. The hormonal influences are among these factors which were shown to have various effects on thymus weight, architecture, and cellularity. Hormones like oestrogen, progesterone, testesterone (Te)[23], ACTH and LH[24], as well as high doses of T_4[25] have been shown to cause different degrees of thymic atrophy accompanied by a marked decline in thymus weight.

The highly significant decrease observed in the present study in the Th.wt. /B.wt. after treatment with OT may be due to the impact of OT on one of the known mechanisms responsible for thymus atrophy. These mechanisms lead either to a deficiency in the number of bone marrow precursors and insufficient migration of these cells to the thymus, an accelerated migration of immature T cells from thymus to the periphery, and / or an enhanced apoptotic and degenerative processes within thymus[26]. The latter mechanism is strongly suggested since the histological findings of the present study support the presence of necrotic cortical and medullary thymic tissues and cell death in the cortex and medulla of the thymus gland in animals treated with OT compared to the control ones. These findings, together with the relative decrease in the C.W. /M.W and the increase in the diameter of HB in response to OT treatment in the present study, provide a strong evidence for a role played by OT in the thymus involution.

It is possible that OT have exerted these effects directly via specific receptors which were described previously[12].

However, indirect mechanism is also suggested, since OT has been shown to stimulate the secretion of LH and ACTH[29] which in turn, cause thymus atrophy[24].

One of the obvious characteristics of thymus atrophy is the severe decline of cortex width which was previously shown to occuur under the effects of hormonal[4] and oncogenic[20] factors. The present study demonstrates that mice treated with OT underwent a decrease in the cortex width of their thymus glands. As an explanation, a partial delymphatization could be the major cause for such a decrease, and this might be explained mostly by the loss of small lymphocytes due either to the great immigration[30] or to the clonal deletion[31] of thymocytes. The histological findings of the present study showed that treatment with OT resulted in necrosis of the thymic cortex as well as desestruction of cortical thymocytes, which may accounted for the decrease in C.W./M.W. caused by OT treatment.

The results have also showed a significant increase in the diameters of HBs in animals treated with each of the three concentrations of OT, and the highest increase was recorded in animals treated with 1 IU OT. The functional role of these structures remains unknown, although their association with cell debris and products of cell death has resulted in speculation that they serve as graveyards for thymocytes[32] or for other thymic

microenvironmental cells, since HBs were shown, in the early stage of involution, to appear relatively large and numerous and formed from hypertrophied and degenerating epithelio-reticular (ER) cells[33]. Our results support the role of these structures as a graveyards to the degenerating thymic cells, since the histological examination have revealed the presence of markedly enlarged Hassall's corpuscles accompanied by necrosis in the medullar tissue, which indicates a severe loss of the medullar cells. Therefore, the present study provides a connection between the increase in the diameter of HB and the decline in the thymus weight resulting from the loss of thymic cells. In conclusion, the present study provides an in vivo support to the hypothesis that OT plays a vital role in the negative selection of T-cells within the thymus.

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HISTOLOGICAL CHANGES OF MICE SKIN UNDER PROTEIN ENERGY MALNUTRITION

Khalida K. Jbara *B.Sc. M.Phil.*, **Nawal M. Abdulla** *M.Sc.* **Kowther K. Hassan** *M.Sc.*

Abstract

Background: Protein energy malnutrition is the most widespread nutritional deficiency disorder that affects growth, maintenance and other body functions.

Objectives: This study was planned to assess the possible effect of protein malnutrition on the histological structure of different layers of mice skin.

Methods: Skin taken from the abdomen of twenty young albino mice aged (8-12) weeks with protein energy malnutrition (PEM) exhibiting skin histological changes. Additional pieces of skin from the abdomen of another 5 sex matched. The control mice where subjected to biopsy under local anesthesia, paraffin sections were made and stained with haematoxyline and eosin and also for collagen, elastic fibers and mucopolysaccharides, and examined by light microscopy.

Results: The results apparently show a variable degree of exaggeration of stratum cornium, with atrophy of stratum granulosum and prickle cells layers was observed. A considerable amount of melanin was found in the basal layer in all cases, there was accumulation of collagen and associated with crowding of elastic fibers. Hemorrhagic areas where observed. The epidermal appendages, hair shafts, hair follicles exhibited atrophy. Sweat glands, sebaceous glands did not show any changes.

Conclusion: The results suggests that overall process of protein and DNA synthesis in skin of malnourished mice are impaired due to the reduction in the number of cells (cell death) rather than a reduction in cell size.

Key words: Mice skin, Histological changes, protein energy malnutrition.

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Introduction

Malnutrition is still a major problem, affecting several organs of the individual body[1,4]. Malnutrition defines by several authors as cellular imbalance between the supply of nutrients and energy and the body demand for them[2], to ensure growth, maintenance and specific function[3].

Protein energy malnutrition the most widespread nutritional deficiency disorder of mankind and many other species[2].

Although protein energy malnutrition affects virtually every organ system[4], therefore this article primarily focuses on its cutaneous manifestations, and because the skin is one of the largest organ of the body which is significantly affecting by protein energy malnutrition[5]. Abteillung and Johann in 1988 observed that animal with protein energy malnutrition have deficiencies of vitamins, essential fatty acids and trace elements, all of which may contribute to the several changes that occurred to the after protein energy malnutrition.

Materials & Methods

Skin specimens were taken from the abdomen of 20 young albino mice aged 8-12 weeks after local anesthesia. These mice were exposed to protein energy malnutrition[7] for four months as shown in table 1. Specimens were fixed in 10% formalin and embedded with paraffin. Sections in about 3-5 microns in thickness were cut and stained with different dyes like haematoxylin and eosin, Massontrichom and Periodic acid Schif reagent, to demonstrate; collagen fibers, elastic fibers, melanin pigment and

Dept. Anatomy, Histology and Embryology, (Medical College of Basrah-University of Basrah)

Address correspondence to Khalida K. Jbara. e-mail: Khalida_habeeb@ yahoo.com

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mucopolysaccharide, and examined by light microscope.
Specimens from 5 control animals fed on normal diet were also Prepared by the same way and examined with the light microscopy. Group 1(Control) fed on normal diet while group 2 (Experimental animals) fed on special formula diet as in table 1.

Table 1: The percentage composition of the diet (%)

Diet Composition	Control group	Experimental Group
Casein	20%	2.5%
Starch	65%	82.5%
Cellulose	4%	4%
Corn oil	4.5%	4.5%
Salt mixture	4.5%	4.5%
Vitamins mixture	2.5%	3%

Results

Results have shown some significance histological changes in the structure of the layers of the skin tissues under investigation, such as hypertrophy of stratum corneum (Figure 1), with atrophy of stratum granulosum and prickle cell layer (Figure 2).

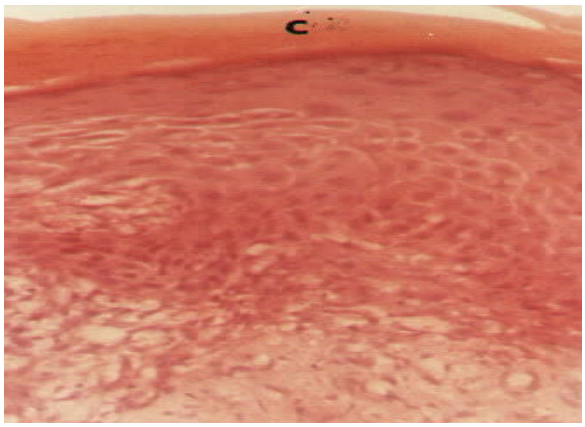


Figure 1: Show hypertrophy of stratum cornium (c) (X 742)

The results also show aggregation of large amount of melanin in the basal layer in all samples (Figure 3), there was also accumulation of collagen fibers as a mass associated with crowding elastic fibers, in some areas of the dermis (Figure 4).
Apparent reduction in the proportion of hair follicles was also observed (Figure 5), the 20th mice which have been examined presented with acute diffused and total hair loss of scalp and other parts of the body, after protein energy malnutrition, hair follicles were found abnormal exhibiting sever atrophy and shaft constriction (Figure 6), most of the hairs examined appear without pigment consistent with the lack of melanin production during the development cycle (Figure 6). Many more broken hairs were found.

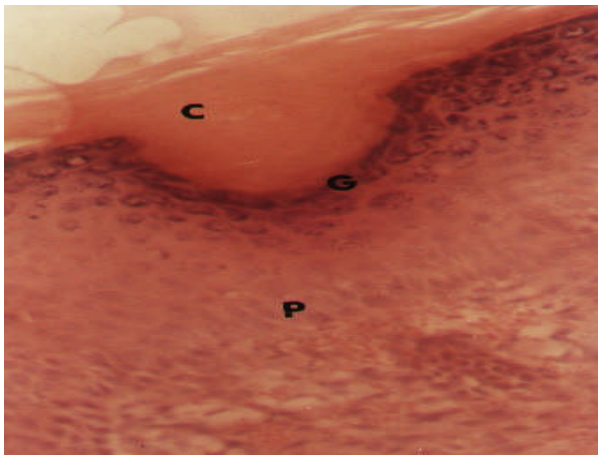


Figure 2: Show stratum cornium (C) with atrophy of stratum (G) and Prickle cell layer (P) (X742)

Large hemorrhagic areas in the dermal papillary layer also seen (Figures 7 & 8) with invasion of some macrophages cells between them. No changes were observed in skin appendages like sweat glands, and sebaceous glands in the present study under the light microscope level (Figure 9). In comparison to the control mice fed on normal free diet (Figure 10 A, B, C).

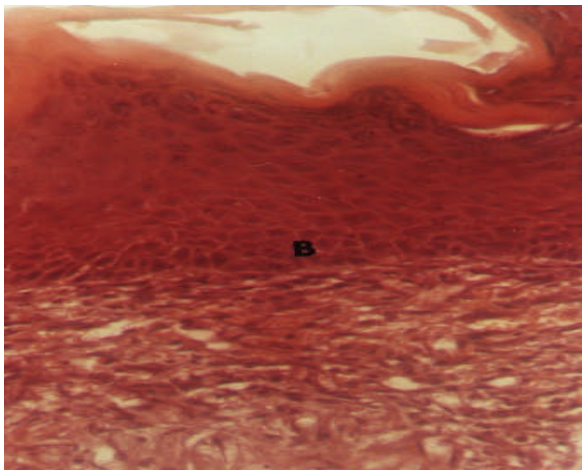


Figure 3: Shows basal layer (B) with large accumulation of melanin (X 742)

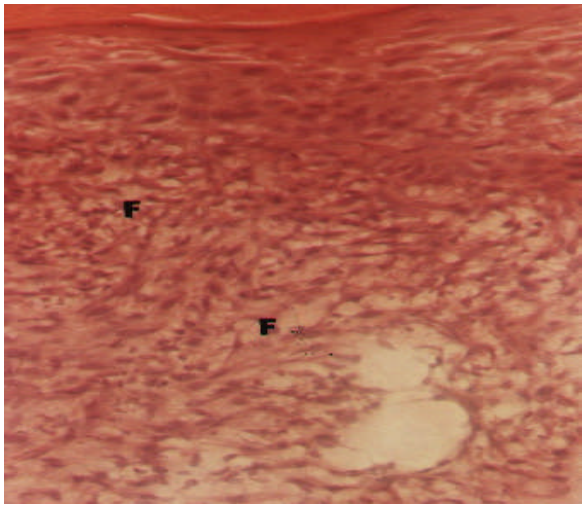


Figure 4: Show accumulation of collagen fibers (F) in same areas of the dermis (X 742)

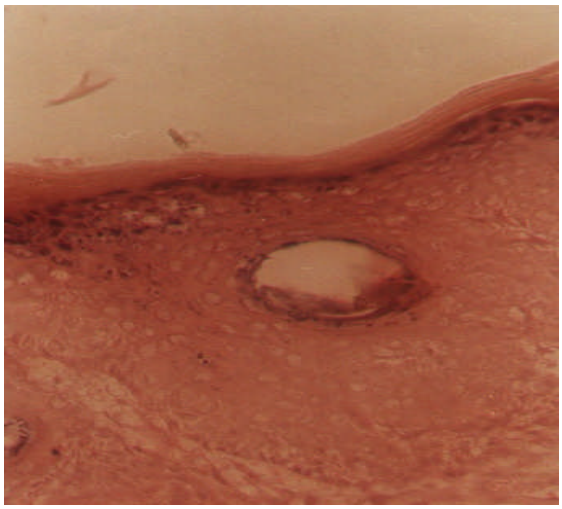


Figure 5: Shows atrophy of hair shafts (HS) and shaft constriction without pigment (X 742)

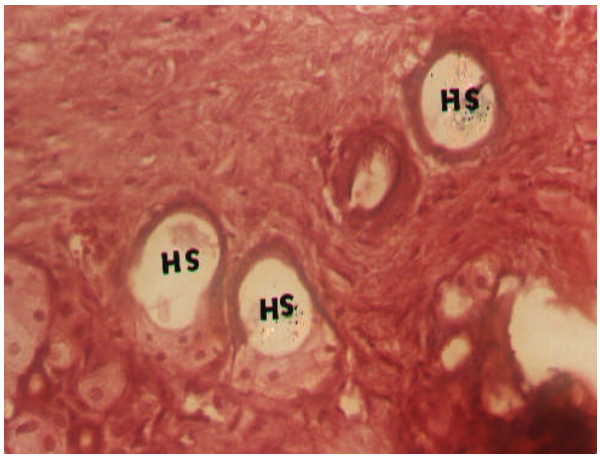
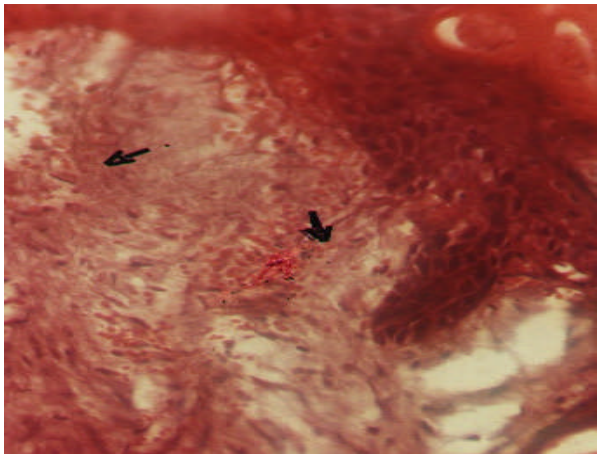
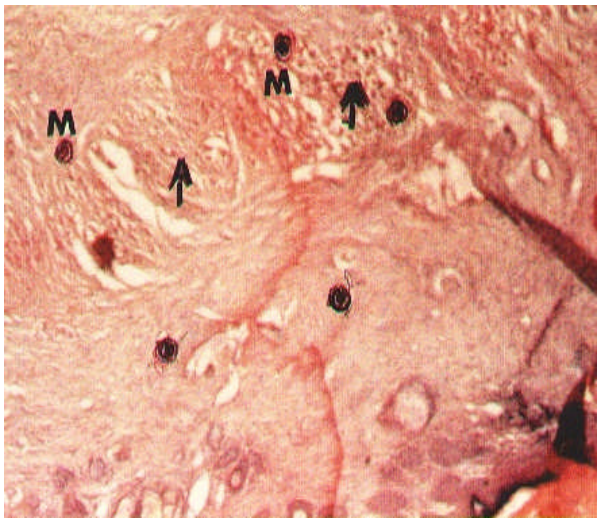


Figure 6: Shows sever atrophy of hair shafts (HS) and shaft constriction without pigment (X 742)





Figures 7 & 8: Shows large hemorrhagic areas (arrows) and invasion of some macrophages cells (m) (X 371)

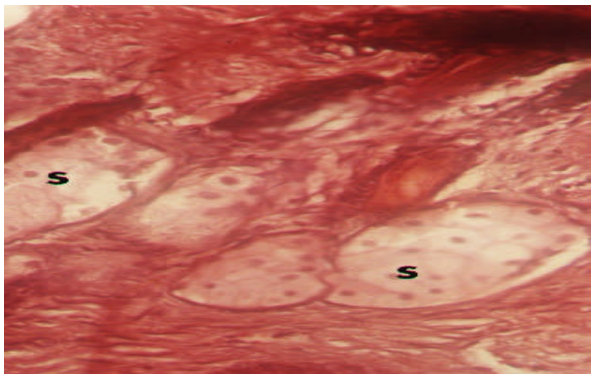


Figure 9: Shows no effect of (PEM) on sebaceous glands (S) (X 742)

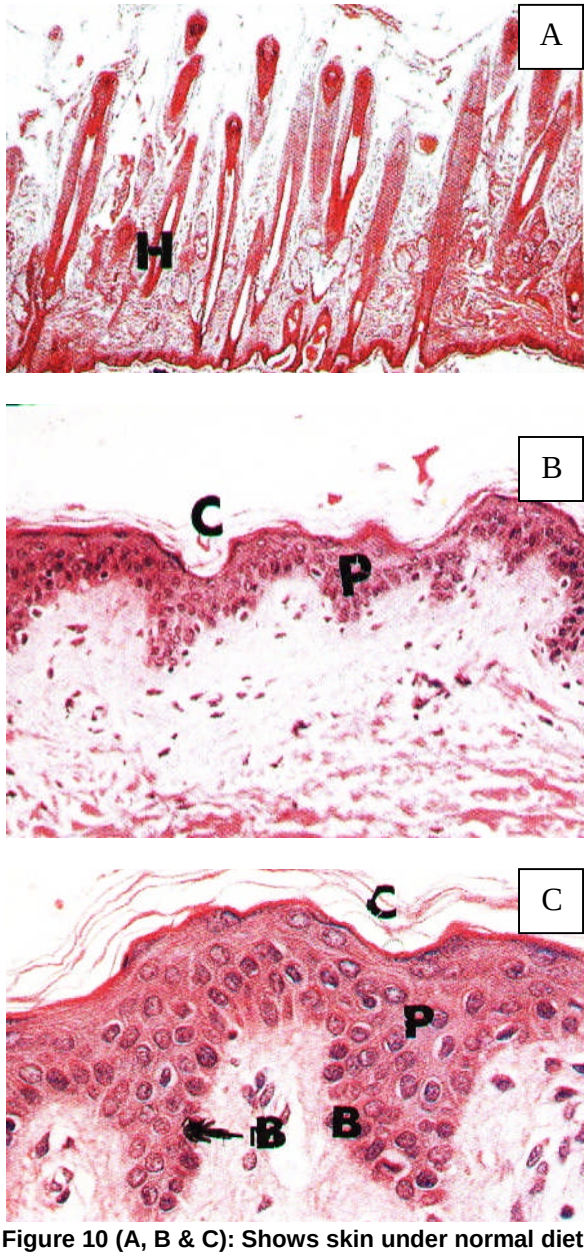


Figure 10 (A, B & C): Shows skin under normal diet (control) A: Skin from scalp with large proportions of hair follicles (H) (X 185) B: Skin from abdomen with normal layers, corneum (C) Prickle cells (P) (X 185) C: High magnification of abdominal skin, stratum corneum (C), Prickle cells (P) and basal layer (B) (X 320)

Discussion

Reducing the protein energy intake in mice to a very low level[7] than that of the control group, led to severe malnutrition with the characteristic histological changes of the skin, like hair follicles, atrophy, hairs loss, hemorrhagic areas, atrophy of stratum granulosum, accumulation of melanin and many other changes, when compare to control mice fed on normal diet. The results however, agreed with the findings obtained by Kaggwa (1986) who showed that restricting the protein diet in rat causing several histological and pathological changes to the skin.

Our findings however agreed with similar findings in human children[9,10]. In Squirrel monkey fed on low protein diet[11]. Prendiville (1992) suggested that hypoproteinemia, occurs more often under condition of protein restriction than protein energy malnutrition. Mclaren et al in 1987[10] suggested that protein malnutrition in human and many other animals like rodents[8], and Avian[12] is consistently associated with lower concentration of serum albumin which causes these changes[8].

Others however emphasized that the skin of malnourished mice contained lower amount of protein and DNA per surface area of the skin than that of the control animals. Nevertheless only protein concentration was lower in malnourished group than that observed in the control group, these results suggest that overall process of protein and DNA synthesis in skin, the skin of malnourished mice are impaired causing skin atrophy, hair loss, hemorrhage, which might be due to a reduction in the number of cells (cell death), rather than reduction in cells size.

It is also possible that mice may be adapted to protein energy malnutrition in which protein synthesis rate would decrease. It could be also explained that mucocutaneous changes occur in some severe cases causes vitamins deficiency state which can also causes skin necrosis[5,6].

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A LIGHT MICROSCOPICAL AND SCANNING ELECTRON MICROSCOPICAL STUDY OF HYPOBLAST FORMATION IN EARLY CHICK EMBRYO

Mahmoud H. Hamash[1] *Ph.D.*, **M. A. Al-Salman**[2] *M.Sc.*

Abstract

Background: The formation of germinal layers in early stages of embryonic development is an essential step and its mechanism is still under study. In chick embryo, which is considered as a model for higher vertebrates' development, the formation of the "hypoblast" is a problem, concerning the source(s) of cells that contribute to it and mechanism of formation of a continuous sheet.

Objective: To study by close observation the details of formation of hypoblastic layer, stage by stage, using light and scanning electron microscopy.

Material & Methods: Chicken eggs were incubated for 5-25 hour at 37.5°C. The embryonic disc of each embryo was examined to decide the specific stage, following Hamburger & Hamilton (H. & H.) staging[1]. The embryonic was fixed and processed to be examined by light and scanning electron microscopes.

Results & Conclusions: The initial growth starts at the caudal margin of the embryonic disc that forms a shield like structure under the surface layer. This shield continues to expand in cranial direction, from stage 1 to stage 3 (H. & H.). This expansion was supplemented by cells invaginating from "ectoblast" and from surrounding marginal zone. The cells gradually contact each other to establish close junctions and hence a continuous sheet.

Keywords: Chick embryo, Blastoderm, Hypoblast.

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Introduction

The origin and the formation of hypoblast germ layer in the chick blastoderm, remain unsettled problem[2-5]. This layer appeared to be of importance for further development[6,7].

Workers in this field had different views. These can be summarized in the following four opinions: This first describes the hypoblast formation by a process of delamination from the epiblast [5]. The second view describes it as a process of invagination or involution around the periphery of the blastoderm into the ventral side[3-5]. The third emphasizes the importance of invagination through the primitive streak[8,9]. The fourth view considers that the hypoblast is totally formed by condensation and flattening of cells migrating from the ventral aspects of the germinal wall around the area pellucida of the blastoderm[10,11].

In addition to the above, a distinct contribution from the caudal (or posterior) germ wall was claimed by others. This contribution was indicated by the appearance of what was known as "embryonic shield" or "Koller's sikle"[11]. This structure was denied by other authors[8,12]. The importance of the hypoblast was re-emphasized especially in establishing the embryonic axis and for there development of the blastoderm[13].

This study was aiming to document the changes in the ventral aspect of the blastoderm in early stages of development, in order to obtain an integrated under standing of the formation of hypoblast.

[1] Dept. Human Anatomy [2] Section of Medical Biology, College of Medicine, Al-Nahrain University.

Address correspondence to Prof. Mahmoud H. Hamash. e-mail: dr-hamash@hotmail.com

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Materials & Methods

Fertilized eggs of chick (Gallus Gallus Domesticus), were incubated for variable time (5-25 hours) according to the stage of study, at 38° C and 70% humidity. At the desired time, the embryo examined through a window in the eggshell. The stage was determined according to the table of Hamburger and Hamilton[8]. The embryonic disc was preliminarily fixed in ova, by applying drops of fixative. The fixative used contains 2.5% Gluteraldehyde in Hanks solution and 5% paraformaldehyde concentration. Tannic acid was added to make a 0.2%. The pH was adjusted to 7.4.

The embryonic disc was then removed from the egg, examined by stereomicroscope, and immersed in the same fixative for several hours and then post fixed in 1% osmium tetra oxide for two hours, then dehydrated by graded ethanol. Specimens to be used for sectioning were infiltrated with propylene oxide for 20 minutes, then in equal mixture of propylene oxide and epoxy resin for one hour, then embedded in Araldite in plastic capsule and left in the oven at 60°c for two days.

These were sectioned, using Riechert ultramicrotome, into 0.5-1 m thickness, and stained with Toluidine blue, to be examined by the light microscope.

Specimens to be used for SEM were transferred after dehydration into 100% acetone for 24 hours at 4° C, and then dried by CO_2 in critical point drying. The specimens were then mounted on studs and gold coated to be examined by Philips 515 scanning electron microscope.

Results

With careful follow up of the expansion of the hypoblast, in the ventral aspect of the blastoderm, it was possible to observe the various sources for the growth of hypoblast. The initial observation by stereomicroscope gave a general view of the blastoderm.

Stage 2:

The embryonic disc is circular with central "translucent" area which is termed: area pellucida. The disc is surrounded by thickened marginal zone, which connect the area pellucida to the surrounding opaque area. The posterior part of the marginal zone is thickened, this is equivalent to with is termed as "germinal wall".

SEM:

Under low magnification, an overview can be obtained for the under surface of the blastoderm, where in the center the light area pellucida is surrounded to the outer side by darker area opaque, where there's crowding irregular cells. In the caudal side the germinal wall consists of crowded cells extending for a distance anteriorly to indicate the beginning of undersurface layer that is the hypoblast (endoblast) (Figure 1).

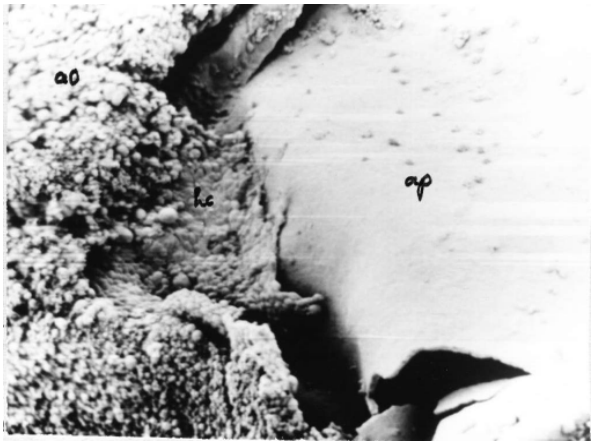

Figure 1: Scanning electron microscope picture for the under-surface of blastoderm at stage (2) seven hours after incubation, showing the area pellucida (ap) and the beginning of grow of hypoblast for the caudal marginal wall (hc) CX 85. Ao = area opaca

By higher magnifications the cells of the germinal wall appear spheroidial, communicating with each other by filopodia & lamellapodia.

Anteriorly the hypoblast cells become more flattened and contact with each other side by side, reducing the spaces between the cells. The uncovered under surface of the ectoblast (of the area pellucida), showed that the cells are in

close contact with each forming a continuous layer. On the under surface of this layer there appear scattered cells in single or in clusters which still in connection with the surface layer and become flatted to different degrees to form scattered islands. (Figure2).

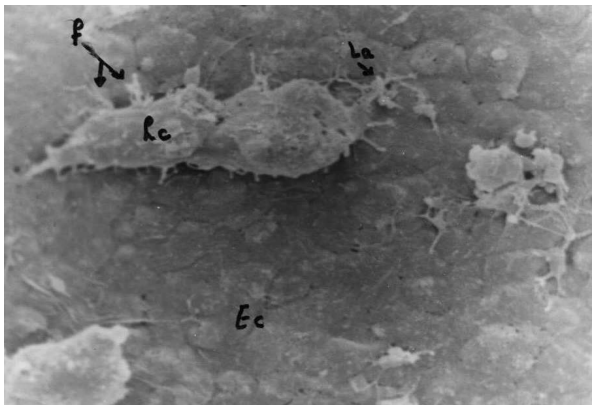

Figure 2: Scanning electron microscope picture for the under-surface of area pellucida showing isolated cell or clusters of cells. Ec = Epiblast cell, F = Filopodia, Hc = Hypoblast cell, La = lamellipodia.

Light Microscopy:

The surface layer appears to be made of closely packed tall or cylindrical cells, that become less tall at the outer region where the this layer overlys the marginal and opaque regions. In the caudal end the germinal wall is made of many layers of cells which are not arranged in stratified layers. The cells which are directly related to the surface layer are relatively small with less yolk granules, but in deeper parts, there are larger collections of cells which are heavily laden dark granules.

The cellular mass forming the germinal wall in caudal part extends interiorly as tapering tongue for a distance reaching about 1/2 the diameter of area pellucida. The cells in this extension which is forming the beginning of hypoblast layer gradually become more flattered and eventually of single cells.

Stage 3:

SEM:

The embryonic disc is still circular, surrounded by area opaque. In the transition zone, there is a groove with a rim like ridge surrounding the area pellucida.

The hypoblast layer extends interiorly to more than the half distance to the rostral margin. On each side there appears a growing sheet from the posterolateral side probably is formed by coalescence of the migrating cells from the lateral marginal sides. The lower surface of the hypoblast layer is formed by flattened cells with are in close contact which each other. It appeared that this is not the only cellular component of the growing layer, but cells appear between this lower layer and the Epiblast layer.

The uncovered regions of the ventral surface of the area pellucida, shows the presence of cells or in clusters, under the coherent layer. In some of these clusters the cells are flattered, and in wide contact with neighbor cells. Some of these flattened cells form islands, in some contact with the growing sheet of hypoblastic layer. (Figure3).

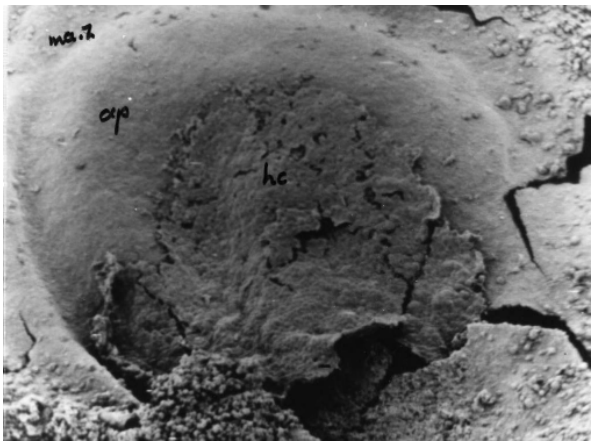

Figure 3: Scanning electron microscope picture for the under-surface of blastoblast at stage (3); 12 hours after incubation showing the growing hypoblastic sheet. Ap = area pellucida, Hc = hypoblast cell, Ma. Z = marginal zone

Light Microscopy:

This stage is characterized by presence of the primitive streak. In longitudinal sections, the surface layer (Epiblast) shows thickening by elongation of cells and increase in compaction. A region in the caudal half of the disc appears with

increase of proliferation and accumulation of cells and becomes multi layered.
The crowded cells extend ventrally to deep level and then stream in anterior direction as a layer of non epithelial cells between Epiblast and hypoblast. These cells of this layer are not separated from the hypoblast, in most instances the two layers are in continuity. Caudal to the region of primitive streak, the cell mass of streak seems to be limited and less populated along the midline, in contrary to the anterior extension and spreading.
The less active epiblastic narrow region is followed caudally by the region which is in the anterior margin of the posterior germinal wall. Here there are scattered clusters of cells underneath the epiblastic layer, which are formed by cuboidal cells at this region. The several layers of cells have similarity with the surface layer and they have less conspicuous yolk granules.
The anterior margin of the germinal wall is in continuation anteriorly with the hypoblatic layer, tapering in its thickness granually.

Stage 4:

SEM:

The area pellucida becomes elongated, indicating an increase in growth rate in antero-posterior axis.
The examination of the under surface of the embryonic disc shows that the hypoblast layer is completed, by growth and fusion with extensions from the lateral sides as well with scattered clusters of cells in the undersurface of the Epiblast, to form a complete continuous sheet (Figure 4). In the midline of the anterior half of the disc there appears a longitudinal bulging, as indication for the head process formation.

Light Microscopy:

The hypoblastic exppears as a complete sheet of more or less flattened cells except in the cephalic part, where the cells around midline are tall or columnar in shape. This part is continuous with marginal zone anterior to the area pellucida.

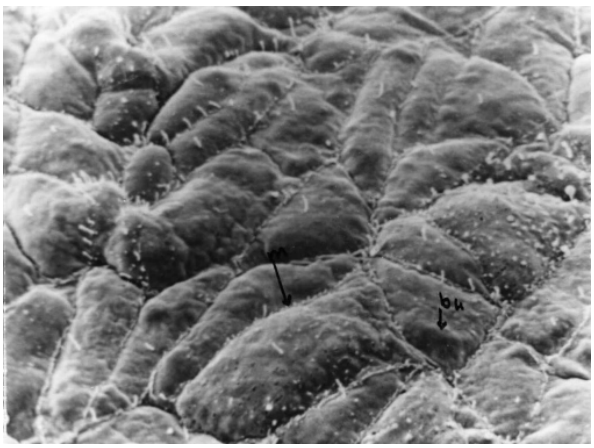

Figure 4: Scanning electron microscope picture for the under-surface of blastoblast at stage (3); 20 hours after incubation showing the completion of hypoblastic later (X136). Bu = bulger, M = microvillie

The mesoderm is more massive, between Epiblast and hypoblast. The cells of this layer some time appear to be mixed with hypoblastic cells or in close association with them.
There are two distinct regions, in which the mesoderm cells are in continuity with Epiblast, these are the region of primitive streak and the region of the Hensen's nods anteriorly (Figure 5).

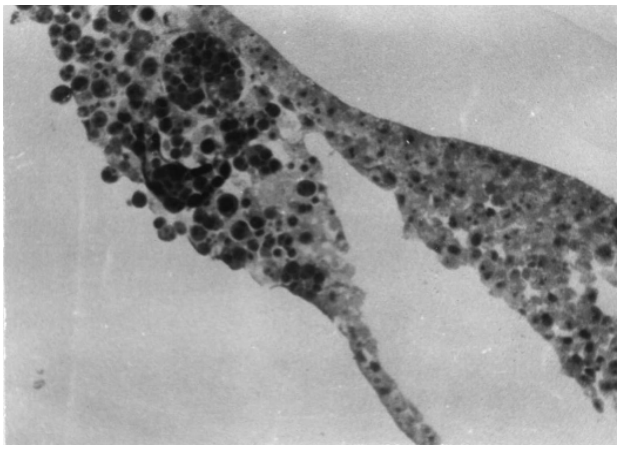

Figure 5: light microscopic micrograph, of alongitudinal section of embryo at stage (4), showing that there are two growth area: rostraly the area of primitive streak, and caudally marginal wall, from which the sheet of hyboblast in extending cephalically. (X120).

These appear as two growth regions. The extension of streaming cells of the mesodermal cells does not reach the

cephalic margin. The area in which the hypoblast is formed by tall cells appears to be devoid of mesoderm cells. Caudal to the region of the primitive streak the cells of the germinal wall are closely related to the Epiblast, and which seem to be continuous with hypoblastic layer anteriorly.

Discussion

The basic description of stage 2 (H. & H.) embryonic disc is in agreement with most previous authors especially Low and co-workers[11] and Vakat[14]. The findings showed the existence of thickening of the posterior "germ wall", and the initial of extension of cells from this germ wall, anteriorly and this may be an indication for the presence of "koller's sikle" which has been denied by some authors[12]. The present finding cannot support the idea, that the thickened germ wall represents a ventral folding of the posterior margin[11,12] rather this area consists of several layers of cells in a disordered arrangement, indicating an active growth region.

At stage 3, there is considerable growth and extension of hypoblast from the posterior side underneath the area pellucida, which is formed by the Epiblast. At the same time, the ventral aspect of Epiblast, shows the presence of scattered clusters of cells, some of these cells are more or less rounded, others are flattened. These clusters appear as islands are the undersurface of area pellucida. Sections studied by light microscopy, show the presence of the primitive streak in the mid line of Epiblast. In the primitive streak there is accumulation of cells, and invagination of some of these cells into the ventral surface of the Epiblast, especially in the region where the hypoblast is well formed as appeared in these sections.

The contribution of migrating cells from the primitive streak into the hypoblast formation was confirmed by many authors[4,8,11], but in this study we could not distinguish the cells from the primitive streak from those from other sources. On other hand this study showed for the first time the presence of two growth regions giving rise to cells streaming in caudo-cephalic direction, one from primitive streak, and the other is from the caudal marginal wall (Figure 5).

At stage 4, the hypoblast formation appears complete, that is the under surface of area pellucida is almost completely covered by the newly formed layer. In sections, it appeared that the hypoblast that is extending caudo-rostraly meets an extension from the anterior marginal wall in restro-caudal direction. This part is characterized by tall cells compared to the rest of the hypoblast.

The changes in cellular morphology during hypoblast formation are shown by scanning micrographs. In early stage, the growing margin of hypoblast, shows some globular cells with some surface appendages at limited contact are as with neigh boring cells. While the cells forming the undersurface, are flattened and in close contact with each other to form a continuous sheet.

In the clusters which are present is increasing number, on the undersurface of epiblastic layer, of the area pellucida, the cells at the beginning are globular or rounded with Microvilli and lamellapodia, gradually change their shapes and become flattened in close contact with each other. Later theses islands of flattened cells with unite with the growing margin of the posterior sheath of the hypoblast.

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RED CELL ION TRANSPORT ALT ERATION IN HYPERTENSIVE PREGNANT

Ibtisam T. Al-Obusi *M.Sc., Ph.D.*

Abstract

Background: A vasospasm and coagulation abnormality of pregnancy-induced hypertension (PIH) has focused on measurement of biogenic amines to explore the pathogenesis of hypertensive disorder. Moreover one of the abnormalities proposed to play an important role in the pathogenesis of hypertension is the ion transport. Class P-ATPase is ion translocases pump that has an important role in maintaining the electrochemical and ion concentration gradients.

Objective: To assess active ion transport alterations and its role in the pathogenesis of the hypertension in pregnancy.

Methods: Seventy-six pregnants were enrolled in this work. Thirty-six were pre-eclampsia (PET) with mean age (30±5) and week of gestation (30±4). Their blood pressure was (155/100). They were on mixed antihypertensive medication (G_2). Twenty pregnants with mean age (29±5) and week of gestation of (28±6) with moderately elevated BP; they were conservatively treated (G_1). Twenty were normotensive pregnant as control group.

Results: ATPase activities measured in µmol pi /mg protein/hr were found to be highly significantly reduced in both hypertensive pregnant ($P<0.0001$ and $P<0.005$). Ca^{+2} ATPase activities were observed to be significantly elevated in (G_1) where as its significantly reduced in PET with respect to control group.

Conclusions: Decreased activity of Na/k ATPase might elevate intracellular sodium, which may lead to increase vascular tone and as a consequence peripheral resistance. Moreover a significant reduction in Ca-ATPase found in PET may result in elevated intracellular Ca+2 which might activate smooth muscle contraction resulting in hypertension. To restore and control blood pressure, those patients have to be treated with antihypertensive drugs that increase ATPase activities, if antihypertensive drugs have to be used. The profound correlation between antihypertensive medication and ATPase activities was discussed.

Key words: Hypertensive pregnants, preeclampsia, ion pumps, ATPases

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Introduction

Na^+/K^+ ATPase distributes ions between the intracelluler and extracelluler space and is resposibale for total body homeostasis.This active ion pumping requires ATP, it moves 3Na ion outward and two K^+ inward for one ATP[1].

Erythrocyte has been widely used as a cell model for demonstrating enzymatic defects in human. Abnormalities in the function of Na^+/K^+ ATPase on cell membranes of erythrocyte have been reported in various diseases and have been proposed to play an important role in pathogenesis of hypertension[2-4] development of proteinuria[2], generation of neuropathy[5,6], diabetes[7], ischemic coronary disease[8], congestive heart failure[9] hyperthyroidism[10] and in renal or liver diseases[11,12].

Calcium plays an important role in the modulation of processes of the cell. The plasma membrane contain Mg^{+2} stimulated Ca^{+2} dependent adenosine triphosphatase (Mg^{+2} + Ca^{+2} -ATPase) responsible for the extrusion of Ca^{+2} ion across the cell membrane against a steep electrochemical Ca^{+2} gradient.

The enzyme contributes to the maintenance of a local concentration of free Ca^{+2} in the cytoplasm to (10^{-7} - 10^{-8}) mole/L[13]. The importance of this enzyme appears when we know that only a small

Dept. Chemistry & Biochemistry, College of Medicine, Al-Nahrain University, e-mail: ibtisam_samn@Iraqi-doctors.com, Msgh1980@hotmail.com, Msalone2002@yahoo.com

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transient increase in the intracellular Ca^{+2} required to bring about smooth muscle contraction[14].

Elevated blood pressure is in most instance associated with an increase in vascular resistance and vascular smooth muscle tone, and is regulated by the concentration of free intracellular Ca^{+2} ion.

Several reports show abnormal cell Ca^{+2} handling in spontaneously hypentensive rat including a reduction in the actions of plasma membrane Ca^{+2}/Mg^{+2} ATPase which my lead to elevated intracellular Ca^{+2} and may link with other membrane changes such as fluidity, binding and phosphoinositol turnover[15].

Hypertensive disorder of pregnancy is a multiorgan system disease involve more than BP. Pre-eclampsia occur when renal involvement leads to proteinuria >300mg/24hr and eclampsia when there is central nervous system involvement.

The exact aetiology is unknown current theories hypothesized in this respect, one of them is altered vascular reactivity and endothelial damage.

As ion transport mechanism is one of important techniques for normal vascular reactivity this work was designed to estimate the transport system and its involvement in pregnancy induced hypertension (PTH).

Patients & Methods

Seventy six pregnant were enrolled in this work. Thirty six were PET with mean age (30±5) years and weeks of gestation (30±4). Their Blood pressure was (155/100). They were on mixed antihypertensive medication(G_2). Twenty pregnant with mean age (29±5) and weeks of gestation (28±6),they were pregnancy induced hypertention (PIH) with moderately elevated BP and conservatively treated (G1), and twenty were normotensive pregnant as control group.

Pre-eclampsia was diagnosed on the basis of blood pressure >140/90 mmHg and proteinuria > 300 mg/24hr.

10 ml of blood where collected in heparinized tubes. Preparation of Erythrocyte membrane was done using method Reinila et al[16] total ATPase activities was measured using Reddy et al[17] Na^+/K^+ ATPase activity measured by method proposed alsewere[3], Ca^{+2} ATPase activity was estimated according to Davis etal method[18].

Total membrane protein by Lowry method[19], inorganic phosphate estimated by ammonium molybdate method.

Statistical analysis

The statistical test used to analyzed the data were: means and standard deviation, student t-test, ANOVA, and correlation coefficient.

Results

Seventy six pregnant were categorized to 20 normotensive as control group, 20 PIH with moderately elevated BP and without medication, and 36 were PET on antihypertensive drugs (aldomate and /or a dalate) G2 (Table 1).

Table 1: Represents groups under study

Subject	Age in years	Weak of gestation	BP mmHg	Treatment antihypertensive drugs
Control normotensive N=20	25±5	28±5	Normal	
PIH (G1) N =20	29±5	28±6	140/90	-----
PET (G2) N=36	30±5	30±4	155/100	Aldomate with or without adalat

Na^+/K^+ and Ca^{+2} ATPase activities of the studed groups were calculated in **µmol pi/mg protein /hr** were shown in table(2)

Table 2: Mean and standard deviation of ATPases in the studied groups

	Normotensive pregnant control N=20	PIH G1 N=20	PET G2 N=36
Na^+/K^+ ATPase	0.557±0.23	0.096±0.008 P<0.0001	0.282±0.106 P<0.005
$Ca+^2$ ATPase	0.041±0.0044	0.166±0.084 P<0.005	0.025±0.02 P<0.05

Total protein of membrane was estimated by Lowry and was found to be between 0.310-0.608 mg/ml.

As shown in table 2, Na^+/K^+ ATPase possess a significant reduction in both hypertensive groups with respect to control group. P<0.0001 & P<0.005 respectively.Moreover a significant elevation of Na^+/K^+ ATPase found in PET when compaired with group one (PIH). Whereas Ca^{+2} ATPase behave differently, as in group one a significant elevation in Ca^{+2} ATPase with respect to control group P<0.005 or with PET group P<0.0001. PET group showed a significant reduction in their Ca^{+2} ATPase with respect to both control P<0.05 or group one P<0.001.

Blood pressure and medication was correlated with Na^+/K^+ and Ca^{+2} ATPase by Spearman Rank Correlation and it was found that both ATPase correlation negatively with blood pressure and with medication (- 0.282; P<0.05) and (- 0.2932; P<0.05) respectively.

Discussion

The data of erythrocyte memberan for hypertensive display various cation pump abnormalities. ATPase activities were compared between the control normotensive pregnant and a two levels of hypertensive disorder of pregnancy.

The firs group was conservatively treated with moderately elevated BP >140/90 while the second group have BP> 155/100 and proteinuria or in other words a progressed form of gestational hypertension with renal involvement.

In human study only blood cells can routinely be used to measure integral protein or Enzymes. Measurements of lymphocyte and platelets are complicated by the fact that the volume of these cells is difficult to assess. Erythrocyte membranes instead are used for such studies[20].

Erythrocyte membrane plays an important role in maintaining normal shape of cell and linking up molecules transportation inside and outside the cell. Erythrocyte produce ATP continuously which keeps up the normal metabolism and function of the red cell.

There are three kinds of ATPase in red cell membrane Na^+/K^+ ATPase, Ca^{+2}-Mg^{+2} ATPase and Mg^{+2} ATPase[21].

The potential importance of Na^+-K^+ ATPase in the generation and maintenance of the resting membrane potential. Many literature focussed several independent lines of investigation on a possible alteration in Na^+-K^+ ATPase function, enzymatic measurement of Na^+-K^+ ATPase activity and in vitro metabolic studies indicating disturbed Na^+-K^+ ATPase function in different disease[1-10].

The activity of erythrocyte membrane Na^+-K^+ and Ca^+ ATPase were studied in spontaneously hypentensive rate[22]. Their observation were reduced Ca^{+2}-Mg^{+2} and Na^+-K^+ ATPase activities.

Some investigations suggested an alteration in the membrane environment in hypertension as primary cause for changes of Ca^{+2}-Mg^{+2} ATPase activity and some of these changes in ion transport act as a markers of membrane abnormalities[22] where as other investigations suggested a role for cellular Mg^{+2} concentration in vascular tone in hypertensive population[20]. In essential hypertensives Resnicket et al[23] found decreased intracellular free Mg^{+2}

concentration in red blood cells as estimated by Nuclear Magnetic Resonance Spectroscopy. Erythrocyte Mg^{+2} deficiency in primary hypertension seems comparatively with few data exist on intracellular electrolyte concentration in vascular smooth muscle cells[24].

An intracellular Mg^{+2} deficiency and possible a defect in intracellular Mg^{+2} transport could play a pathogenic role in the development of primary hypertension and/or in induced one.

On the bases of expensimental data the mechanisms underlying the Mg^{+2} induced vasodilation may be a modulation of response to vasopressor hormone. An interaction with cellular Ca^{+2} handling which is supported by three lines of recent evidence: first cellular Mg^{+2} concentration can influence Ca^{+2} metabolism of vascular smooth muscle by changing the Ca^{+2} influx through the plasma membrane. Second change in the extracellulr Mg^{+2} concentration induce inverse changes in Ca^{+2} content of vascular smooth muscle and exchangeable Ca^{+2}[25] and third a decrease in free intracellular Mg^{+2} concentration results in diminished membrane Na^+/K^+ ATPase and Ca^{+2} ATPase activities[26] which will leads to in an increase in Na^+/Ca^{+2} exchange and intracellular Na^+ and Ca^{+2} concentration . Cellular Ca^{+2} overload is a major impairment that leads to the loss of membrane integrity, membrane alteration and increased osmotic fragility[27].

Interaction between Mg^{+2}, Ca^{+2} and K^+ channels Mg^{+2} interferes with voltage-gate Ca^{+2} channels and with various K^+ channels (delayed gate K^+, K^+-Ca^{++} channels and particularly K^+ ATP channels in vascular smooth muscle and endothetial cells[28].

In clinical cases in which hypertension is sensitive to sodium Chloride balance a decrease in Na^+/K^+ ATPase activity is believed to produce vasoconstriction and sodium retention[29].

A reduction of sodium pump activity in vascular smooth muscle, or in ability to increase its activity in face of greater sodium influx into vascular smooth muscle cells has been postulated to explain hypertension. Such reduction in this enzymatic activity may reflec an alteration of the sodium pump itself, or may be mediated by circulating inhibitors or may result in alteration of endothelium which modulate vascular response to its natural antagonist[30]. In present data the activities of erythrocyte ATPase were studied and the observed decrease in Na^+/K^+ ATPase in both hypertensive pregnant could reflect the accumulation of Na^+ intracellularly, this accumulation could increase Na^+/Ca^{+2} exchange or could partially inforce or enhance the Ca^{+2}-ATPase activity as evident by significant increment found in Ca^{+2}-ATPase in group one. The more pronounce decrement in Na^+/K^+ ATPase the more the activation in Ca^{+2}-ATPase (0.09 versus 0.5 for normal Na^+/K^+ ATPase) and (0.166 versus 0.04 for normal Ca^{+2} ATPase).

This picture is not the same in PET where they have a more and aggressive elevated BP, renal involvement and they where on antihypertentive medication. As these are amultifactorial causes the Na^+/K^+ ATPase partially restored (0.282 versus 0.09 for group one and 0.5 for normal). This partial improvement could not be explained as an improvement in BP as reported elsewhere[30] because the BP of group two is much more increased with respect to group one.

The severe reduction in Ca^{+2}-ATPase found in PET (0.02 vrs 0.166 for G1 and 0.04 for normal) could play a role in resorting the partial activity of Na^+/K^+ ATPase. Moreover antihypertensive medication was given to group two but not group one. Spearman Rank Correlation was used to test the effect of drugs on ATPase and it was found to be negatively correlated specially for Ca^{++} ATPase.

Golik et al[30] observed that the activity of Na^+/K^+, Ca^{+2}-Mg^{+2} ATPase activities were enhanced upon treating with captopril and enalapril drugs.

It could be the time now for testing a safe antihypertensive drug that improve the altered ATPase activities, restore the intracellular concentration of Na^{+}, Ca^{++} and Mg^{+2} which will decrease the vascular tone and the consequence peripheral resistant and smooth muscle contraction in such PET patient for prevention of further progress of the disease and to prevent eclampsia.

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INVESTIGATION OF SOME MAJOR AND MODIFIED NUCLEOSIDES IN PATIENTS WITH ACUTE LEUKEMIA

Alaa K. Mohammed[1] *Ph. D*. **Sarmad B. Dikran**[2] *Ph.D.* **Kawther A. AL-Zubaidi**[2] *Ph.D.*

Abstract

Background: A recently developed high-performance liquid chromatography method permits quantitative measurement of low levels of modified nucleosides in biological fluids. The nucleosides inosine, guanosine and pseudouridine as well as the bases hypoxanthine in serum are thought to be more useful than those in urine as tumor markers because they are not influenced by other factors.

Objectives: To determine some nucleosides and their basis by HPLC and correlate their concentrations in serum with acute leukemia.

Methods: Forty-three individuals were included in the present study, 9 with acute myelogenous leukemia (AML) {age range 9-45 years}, and 13 with acute lymphoblastic leukemia (ALL) {age range 7-58 years}. They were compared with 22 matched healthy control subject {age range 8-60 years}, and did not receive any medication. Serum compound were estimated using reversed phase high performance liquid chromatography (RP-HPLC)

Results: Results of this study revealed that the mean inosine, guanosine and hypoxanthine level in the sera were elevated in the leukemic in comparison the normal subjects, and the level of serum pseudouridine, a degradation product of transfer ribonucleic acid, was significantly elevated in those patients with acute melogenous and acute lymphoblastic leukemia when compared with the normal control.

Conclusion: Therefore, we may propose that the assay level of these compounds in sera of leukemic patients might be useful to predict the effectiveness of therapy in very beginning stages and further investigation needed to better assess the utility of these molecules as a tumor marker.

Key words: Serum modified nucleosides; pseudouridine and leukemia.

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Introduction

Numerous studies have documented the occurrence of increased level of major and modified nucleosides and their bases in biological fluids of cancer patients[1-4].

Increased tRNA methylase activity is characteristic of malignant tissue, and tumor bearing animals when compared to their normal tissue counterparts[5-8]. When RNAs are degraded, modified nucleosides are excreted in the urine as an intact molecule because they have no salvage pathway[9]. The abnormal increase of major and modified nucleosides in the biological fluids of cancer patients has been attributed to a higher turnover rate of the tRNA in the tumor tissue destruction[10].

Several reports have been published on the usefulness of the serum and urinary nucleosides detection by High Performance Liquid Chromatography (HPLC) system as diagnostic markers with potential for early detection of cancer for monitoring of effectiveness of the therapy[11] and for diagnosis of population of risk, such as asbestos workers[12] and patients with AIDS[13].

Although the content of nucleosides and their bases in serum has been reported to be much lower than that in urine, but in spite of its low serum level, it seems to be reliable for the estimation since they are less affected by exogenous factor than those in urine[14].

The present study examines the serum levels of some major and modified nucleosides in patients with acute leukemia and lymphoma to determine

[1]**Department of Chemistry, College of Education (Ibn-Al-Haitham), University of Baghdad** [2]**Department of Pharmaceutical Science, College of Pharmacy, University of Tikrit.**

Address Correspondence to: Dr. Alaa. K. Mohammed, E-mail: Alaakareem@maktoob.com

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their potential value in monitoring disease as diagnostic biochemical markers.

Materials & Methods

Chemicals: Guanosine, inosine, pseudouridine and hypoxanthine were purchased from Supelco chemical company (U.S.A.). All reagents used were of A.R. grade quality.

Apparatus: The HPLC equipments were obtained from Shimadzu-Corpo.LTD, Japan, comprises of (LC-6A), dual head reciprocate pump. A variable wavelength UV-detector (SPD-6AV) mounted at 254nm and linked with (C-R4A) Chromatopack data processor, capable of processing data and printing chromatogram on the same recording chart. Automatic injector system (SIL-6A) fitted with 50 ul sample loop was used for sample injection.

Patients and controls The sera of forty-three patients, including "AML" and "ALL", derived from the medical center (Baghdad) were included in this study; they were divided into three groups:

A: Acute myelogenous leukemia (AML), this group included eight patients (3 females, 5 males) with age ranged between (9 - 45) years.

B: Acute lymphoblastic leukemia (ALL), this group included thirteen patients (4 females, 9 males) with age ranged between (7 - 58) years.

C: Normal controls comprised of 22 normal healthy persons (6 females, 16 males) with age ranged between (14 - 60) years.

The diagnosis of the disease was based on hematological examination in all cases, and all patients have normal renal function and were free of bacterial infection at the time that the serum collected.

The blood was collected from each person at the initial diagnosis before induction therapy.

Sample preparation The leukemic venous blood sample was drawn aseptically into siliconized tube without anticoagulant and allowed to clot for 30 minutes at room temperature, then centrifuged at 800 r.p.m. for 15 minutes. Serum (500 µl) was diluted with equal volume of (0.4 M) sodium potassium phosphate buffer (pH = 8.4) and centrifuged at 1000 r.p.m. for 30 minutes. The centrifugation obtained was kept at -20^{o}C until the RP-HPLC analysis. Chromatographic conditions The UV-absorbing compounds and serum samples were separated on commercially available column (25 cm x 4.6 mm i.d.) zorbax / C18 (water associates comp) packed with 5 µm particle size consist of porous silica to which a mono layer of octadecyl trichlorosilane is chemically bounded. For the separation, linear isocratic elution mode of 0.02 M K_2HPO_4 buffer (adjusted to 5.5) was used as a low strength eluent mixed with 10 % acetonitrile as a high strength eluent. All eluents were degassed by purging with helium.

The flow rate of the mobile phase was 1.4 ml.min^{-1} and the column temperature was kept at 35^{o}C using Shimadzu CTO-6A column oven. The whole system was controlled by Shimadzu SCL-6A controller.

The quantitative analyses of compounds have been carried out at 254 nm by comparison the peak height of each of them with the corresponding peak height of the standard under the same chromatographic conditions.

Detection limits and linearity of response

The detection limits for all compounds were found to be in the range of 15-30 pmol. Calibration graphs of peak area versus concentration and peak height versus concentration were constructed and found to be linear in each case for the studied concentrations in the range of 60 pmol. to 450 pmol. per injected volume.

Results

For the separation of the major and modified nucleosides and their bases, a

reversed phase mode of HPLC was used; chromatographic conditions were optimized in order to achieve interference free separation of the compounds under study in the serum matrix. Figure 1, illustrates the separation of the standards of the investigated compounds.

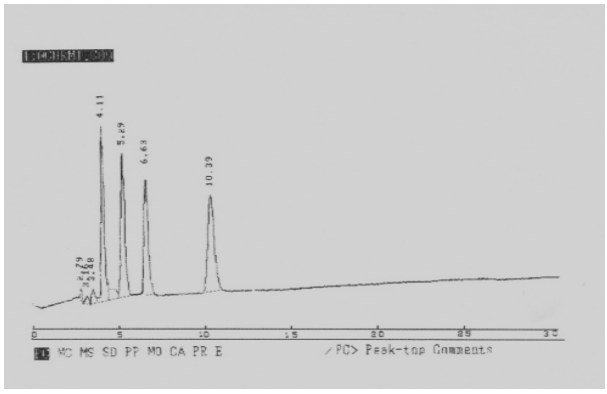

Figure 1: Separation of standards of the studied compounds; (1) Pseudouridine, (2) Hypoxanthine, (3) Guanosine, (4) Inosine.

Initial assignment of solutes was first tentatively identified by means of their retention behaviors and co-chromatography with reference compounds. The chromatograms of sera samples form patients of "ALL" and "AML" (Figure 2 and Figure 3) were compared to those of normal subjects (Figure 4), and were generally characterized by an increase in the levels of pseudouridine, inosine, guanosine, and hypoxanthine.

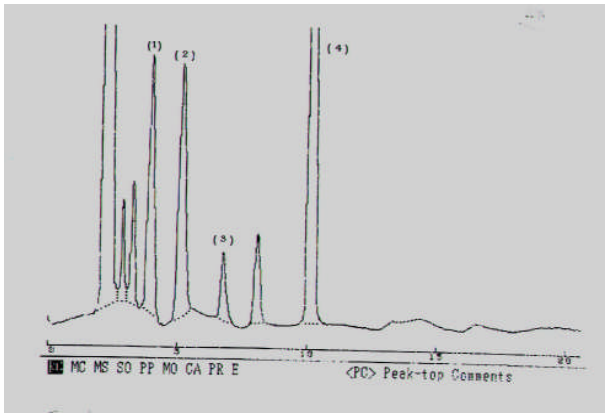

Figure 2: Chromatogram of plasma sample obtained from an ALL patient.

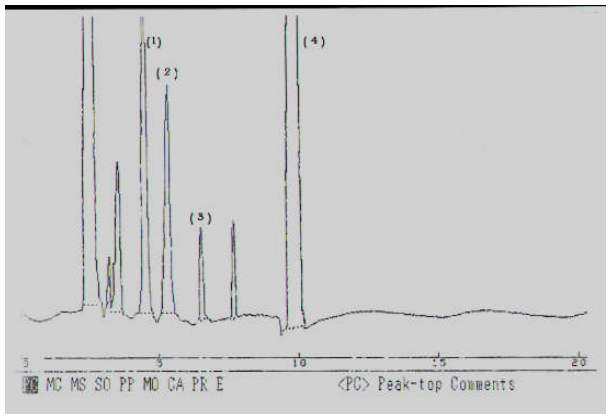

Figure 3: Chromatogram of plasma sample obtained from an AML patient.

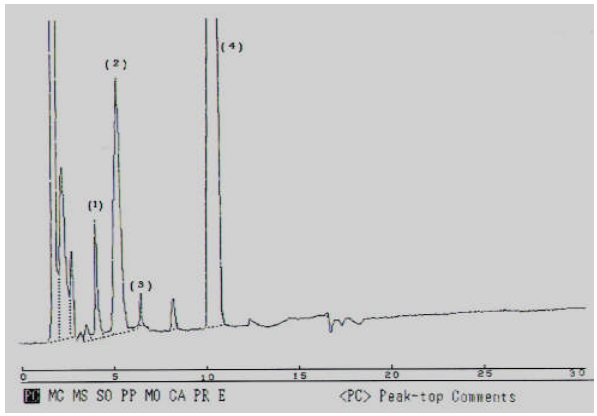

Figure 4: Chromatogram of plasma sample obtained from a normal patient

On the other hand, the UV profiles chromatograms of a deproteinized serum from normal subjects for compounds were very consistent for the group of normal subjects. The only peak, which showed considerable variation within this group, was the inosine. A tabulation of the average concentration and rang (mean ± 2SD) of the compounds identified in the sera of twenty-two normal subjects (16 females and 6 males) is given in table 1.

Table 1: **The average concentrations of identified compounds in the sera of normal subjects**

Compounds	Mean ± SD µmol./ l.	Range(mean ±2SD)
Inosine	0.52±0.24	0.04-1.00
Pseudouridine	2.20±0.32	1.56-2.84
Guanosine	0.19±0.09	0.01-0.37
Hypoxanthine	2.01±0.46	1.09-2.93

The results of the serum inosine level measurement are summarized in table 2.

Table 2: Serum levels of inosine in normal and patients with leukemia and lymphoma

Disease	No.	Mean ± SD µmol./ l.	P value
AML	8	3.55 ± 1.93	P<0.005
ALL	13	2.96 ± 1.12	P<0.005
Control	22	0.50 ± 0.28	-

AML = Acute Myelogenous Leukemia.
ALL = Acute Lymphoblastic Leukemia

The mean inosine concentration in twenty-one "AML" and "ALL" patients sera were found to be 3.25 µmol. liter^{-1}, while the corresponding level in twenty-two healthy subjects was 0.50 µmol. liter^{-1}. This increase in inosine level was statistically significant (P < 0.005). As different types of diseases may specifically possess different levels of certain major and modified nucleosides, separate calculations were made for each type.

The mean value of inosine in normal serum samples was found to be 0.50 µmol.liter^{-1} and those for "AML" and "ALL" were amounted to 3.55 and 2.96 µmol.liter^{-1} respectively. This increase in inosine level was statistically significant (P < 0.005). The results also show that in 37 % of the patients with "AML", serum level of inosine exceed the mean ± 2 SD of the healthy (1.06 µmol. liter^{-1}), whereas only two patients with "ALL" (16 %) showed inosine level above this threshold.

Table 3, presents the results obtained for the measurements of serum pseudouridine level.

Table 3: Serum levels of pseudouridine in normal and patients with leukemia and lymphoma

Disease	No.	Mean ± SD µmol./ l.	P value
AML	8	3.91±1.08	P<0.005
ALL	13	3.21±1.22	P<0.005
Control	22	2.20±0.32	-

AML = Acute Myelogenous Leukemia.
ALL = Acute Lymphoblastic Leukemia

The mean of pseudouridine concentrations in twenty-one patients with "AML" and "ALL" was found to be 3.45 µmol.liter^{-1}, while the corresponding level in twenty-two healthy subjects was 2.2 µmol.liter^{-1}, this increase (56%) in these patients was highly significant (P < 0.005).

The mean value of pseudouridine in "AML" serum was found to be 3.91 µmol.liter^{-1}, this (77 %) increase in pseudouridine level was statistically significant (P < 0.005), while the mean pseudouridine level was (45 %) higher in sera of "ALL" (P < 0.005).

The most intriguing results were found in the majority of the chromatograms for patients with "AML" and ten of the chromatograms for patients with "ALL", in which an elevation in pseudouridine level above the mean ± 2 SD is observed when compared to those of normal subjects.

The results show that inosine and pseudouridine might be clinically useful as complementary markers to monitor the disease status of the patients with leukemia and lymphoma by hematological examination.

Table (4) illustrates the results of the guanosine analyses.

Table 4: Serum levels of guanosine in normal and patients with leukemia and lymphoma

Disease	No.	Mean ± SD µmol./ l.	P value
AML	8	0.58±0.23	P<0.005
ALL	13	0.33±0.17	P<0.005
Control	22	0.19±0.09	-

AML = Acute Myelogenous Leukemia.
ALL = Acute Lymphoblastic Leukemia

The mean value of it in the sera of normal subjects was 0.19 µmol.liter^{-1} and that of "AML" and "ALL" were amounted to 0.58 and 0.33 µmol.liter^{-1} respectively.

This increase in guanosine level was statistically significant (P < 0.005). Striking differences were found in three chromatograms for patients with "AML" (37 %) and four chromatograms for

patientswith "ALL" (31 %), which showed an elevation in the guanosine level above the mean±2 SD when compared to those normal.

Table 5 shows the level of hypoxanthine in two categories of patients under study in comparison with normal.

Table 5: Serum levels of hypoxanthine in normal and patients with leukemia and lymphoma

Disease	No.	Mean ± SD µmol./ l.	P value
AML	8	53.42±48.22	P<0.005
ALL	13	45.03±32.20	P<0.005
Control	22	2.01±0.46	-

AML = Acute Myelogenous Leukemia.
ALL = Acute Lymphoblastic Leukemia

The mean value of hypoxanthine in normal serum was found to be 2.01 µmol.liter^{-1} and that of "AML" and "ALL" sera amounted to 53.42 and 45.03 µmol.liter^{-1} respectively. This increase in hypoxanthine level was statistically significant ($P < 0.005$).

Discussion

The objective of this investigation was to evaluate the serum level of these compounds in patients with Leukemia and Lymphoma then to correlate and used them for the initial diagnostic purpose. The concentration levels of inosine, pseudouridine, guanosine and hypoxanthine in the sera were considered to be elevated when they exceed the normal mean value by 2 SD .It was suggested that the high levels of these compounds is related to the disease stages.

One of the hypothesis proposed to explain the elevated level of inosine suggested that this may be due to the decomposition of large amounts of inosine monophosphate (IMP) initially formed from hypoxanthine via the salvage pathway of hypoxanthine guanine phosphoribosyl tranferase (HGPR Tase). Moreover, the direct synthesis of inosine from hypoxanthine via purine nucleoside phosphorylase may be enhanced[15]. Thus, it is postulated that the individuals, in whom the slavage pathway of hypoxanthine is extremely active, experience a more serious development of the disase.

Pseudouridine is produced as a result of degradation of tRNA, and since it is neither metabolized nor incorporated in tRNA formation, its level consequently elevated and considered to reflect rate of tRNA turnover[16]. Accordingly, the increase in pesudouridine level could be possibly useful as a mean for determining tumor response to therapy and as a valuable marker for monitoring the course of patient during treatment.

It has been found that the level of pseudouridine excretion drops down to normal value after commencement of chemotherapy and remains normal as long as patient is in remission, this was found in Brukitts lymphoma and T-cell acute lymphocytic leukemia[17]. The elevated levels for not only ionsine and pseudouridine but also guanosine and hypoxanthine in the biological fluids of cancer patients suggest the increased tRNA turnover as a responsible factor[18].

From these observations, assay level of these compounds in biological fluids of patients with leukemia and lymphoma might be useful to predict the effectiveness of therapy in very beginning stages and to better assess the utility of these molecules as a tumor markers.

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EFFECT OF LEVAMISOLE ON THYMOCYTE APOPTOSIS

Israa F. Al-Samaraee *M.Sc, Ph.D.*

Abstract

Background: Levamisole is an antihelmenthic drug; recently it's used as immunomodulator, in many diseases, as nephrotic syndrome, and also used as adjuvant in colorectal cancer but its mode of action is unknown. Apoptosis is an important physiological process that involves the deletion of specific cells, characterized by specific DNA cleavage.

Objectives: To study the effect of levamisole on thymocytes, the probable action of this drug in induction of apoptosis, and its relation to the CaATPase enzyme activity.

Methods: 12 rats were involved in this study. 6 used as control, and 6 were treated with levamisole 2mg/kg for 6weeks.Estimation of Ca ATPase enzyme activity, were assessed in blood and thymic tissue. Sections were examined by light microscopy and electron microscopy.

Results: There were apoptotic changes in group receiving levamisole in more than 60% of cells. The activity of Ca ATPase in tissue and blood of rats prior and post levamisole supplement, showed significant changes in group receiving levamisole P>0.05.

Conclusion: levamisole can induce apoptosis in rat thymocyte probably by decreasing Ca ATPase enzyme activity.

Key words: Apoptosis, Ca ATPase, levamisol.

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Introduction

Levamisole is antihelmenthic drug, used in muscle, liver, kidney and fat of sheep, pigs and poultry; recently it's used as immunomodulator, in many diseases, like nephrotic syndrome[1], and also used as adjuvant in colorectal cancer but its mode of action is unknown[2].

Apoptosis is an important physiological process that involves the deletion of specific cells in a controlled and timely manner. The characteristic of apoptosis is DNA cleavage by calcium dependent nuclease[3].

Calcium ion serves as the main second messenger in all cell types including thymocytes and lymphocytes. The cytosole free calcium concentrations are maintained at a very low level (50-150nM). The changes in intra cellular Ca^{+2} concentration are essential for normal cellular activities which are concerned with cell development, mitotic activity and immune response[4].

Ca homeostasis in cells is a complex mechanism and maintained through transporter located in cell membrane. The calcium efflux from excitable cell occurs through 2 main systems: Electrochemically driven Na/Ca exchanger with low Ca^{+2} affinities, and a plasmalemmal Ca ATPase with a high Ca affinity. In non excitable tissue like leukocyte in general the activity of Ca ATPase is the sole system responsible for the extrusion of Ca ions outside the cell. Thus Ca ATPase is considered a fine tuner of cytosolic calcium ion concentration[4]. The proposed action of Ca in induction of apoptosis in autoimmune diseases for example is through Chemokine-induced Ca^{+2} mobilization which is likely occurs via intracellular inositol trisphosphate (IP3) receptors, because in many cell types the G protein-coupled chemokine receptors are known to activate phospholipase Cb2, and in turn, generate IP3[5]. membrane phospholipid Diacylglycerole and Inositole triphosphate act as second messenger and lead to release of Ca^{+2} from internal

Dept. Clinical Physiology, College of Medicine, Al-Nahrain University
Address correspondence to Dr. Israa F. Al-Samaraee, P.O.Box 14222, E-mail: col_med_nahrain@yahoo.com
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stores the increased Ca^{+2} inside the cells lead to increased activity of enzymes like ATPases endonuclease proteases and this will induce apoptotic process[6,7]. Indeed, a sustained increase in intracellular Ca^{+2} accompanies T and B cell receptor signaling and is necessary for gene activation, cellular proliferation, and antibody secretion[5], while apoptotic body engulfment and processing are accompanied by rise in intracellular Ca^{+2} and are dependant on internal Ca^{+2}. Disturbances in Ca^{+2} passive permeability and intracellular Ca^{+2} homeostasis are an apoptosis trigger for example in lethally irradiated thymocytes[8]. Spinozzi et al have found that a pair of mitogenic anti-CD2 mAb provoked a dramatic rise in intracellular Ca^{+2} concentrations that were almost entirely sustained by extracellular fluxes, and the inhibition of membrane Ca/Mg ATPase. The resulting endonuclease activation was able to induce DNA fragmentation, and hence apoptosis[9]. Choi et al[10] observed that cells rapidly undergo massive apoptosis following culture with specific inhibitor of the Ca ATPase, which means that increase Ca^{+2} inside the cell will enhance apoptosis[10].

Hakansson[11] revealed that a component of milk in a particular physical state--multimeric alpha-lact-albumin is a potent Ca^{+2} -elevating and apoptosis inducing agent with broad, but selective, cytotoxic activity. These findings can suggest a design of antitumor agents by inducing rise in intracellular Ca^{+2} which induce apoptosis[11].

Methods

12 aged-matched albino rats (Rattus norvegicus albinus) were involved in this study. Rats aged 3 weeks weighing 40-50 gm. 6 rats used as control, and 6 were treated with levamisole 2mg/kg for 6weeks. Rats were anesthetized by ether then blood samples were aspirated from rats for the estimation of Ca ATPase enzyme activity, thymus was excised then animals were sacrificed.

Thymus was dissected, sections examined by light microscopy (after staining with touludin blue) for morphological features of thymocyte apoptosis. The ultrastructural features of thymic tissues were studied by electron microscopy according to Reynolds method[12].

The rest of the tissues were processed for the estimation of Ca ATPase enzyme activity, In both groups (control and study groups) the activity of Calcium ATPase were measured according to Davis 1981 i.e. prior to levamisol supplement and after levamisol supplement[13].

Results

The results can be divided to cellular changes indicated by morphological changes of thymocyte (apoptotic features) and enzymatic activity.

Cellular changes: histological sections were stained by touludine blue and examined by light microscope, these revealed: Apoptotic changes in group receiving levamisole in more than 60% of cells. These changes include: chromatin condensation, fragmentation of nucleus, c-shaped nucleus as shown in figures 1 and 2.

The percent of apoptotic cells in the treated group as compared to control group was significantly different. Sections examined by electron microscope confirm the diagnosis of apoptosis as shown by figure 3.

Enzymatic changes: The activity of Ca ATPase was measured in tissue and blood of rats prior to and after levamisole supplement, the activity of the enzyme was significantly reduced in group receiving levamisole $P>0.05$, as shown in table 1.

Table 1: The changes in Ca ATPase activity in control and treated groups

	control	Pre-treatment (control)	Post-Treatment	P-value
Tissue/CaATPase/ mmol/mg/min	1.5±0.32	1.5±0.32	0.67±0.04	0.05
	control	**Pre-treatment**	**Post treatment**	**P-value**
Blood/CaATPase/ mmol/mg/min	1.039±0.141	1.02±0.52	0.633±0.032	0.05

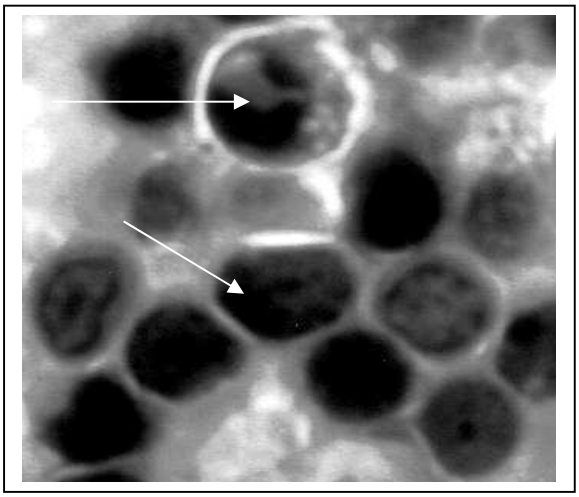


Figure 1: Apoptoic thymocyte show condensed fragmented chromatin, c-shaped chromatin, light microscope/ toludine blue.100x

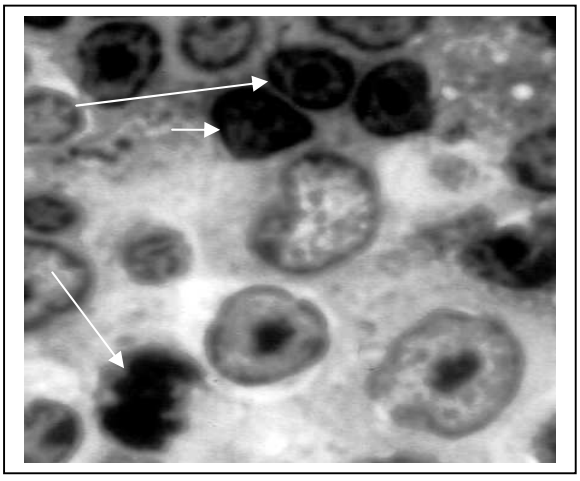
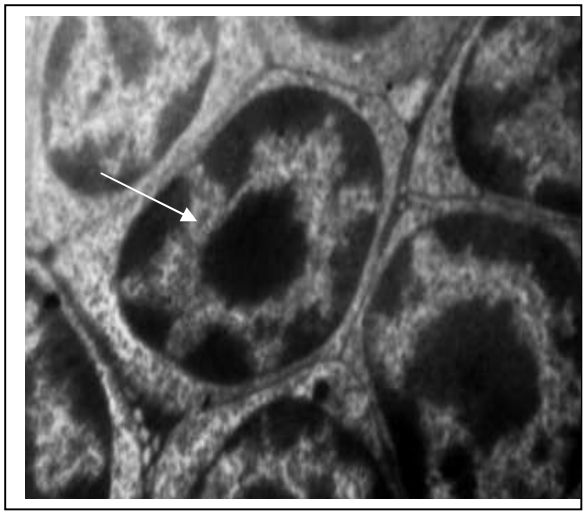


Figure 2: Normal thymocytes/thin arrow. Apoptotic thymocyte thick arrow. light microscope toludine blue 100x



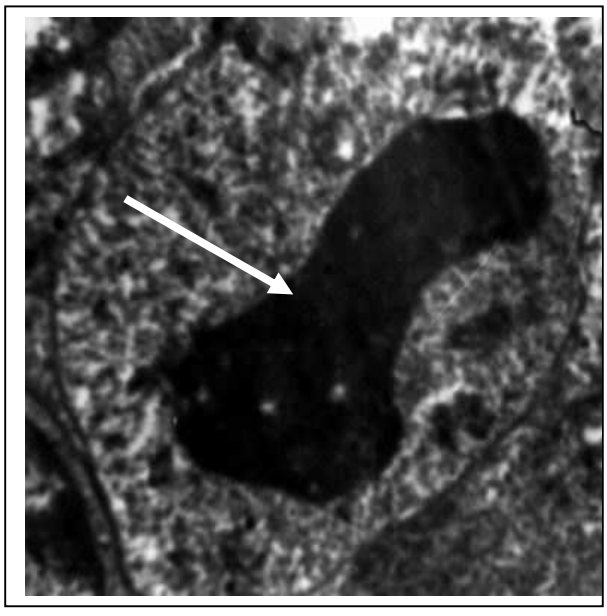


Figure 3: (up) Normal thymocyte (thin arrow); (down) Apoptotic thymocyte with condensed chromatin, intact mitochondria (thick arrow); Uranyl acetate and lead citrate/EM

Discussion

Different studies had shown that inhibition of the Ca^{+2}/calmodulin-dependent phosphatase calcineurin with perfluoreperazine dimadeate, a calmodulin antagonist, or cyclosporin A, a specific inhibitor of calcineurin, reduced the magnitude of apoptosis in leukemia cells. These findings provided an evidence for a Ca^{+2} dependent apoptotic signals in human ALL cells. The antileukemic potency of the dual function of calcium mobilizer/PKC (protien kinase C) inhibitor calphostin C, induce apoptosis in human ALL cells, prompted the hypothesis that the PKC inhibitory function of calphostin C is also contributes to its Ca^{+2}-dependent antileukemic activity. The Ca^{+2} signal is an initiator of apoptosis, and once initiated, the execution of apoptosis does not depend on the continued exposure to calcium-mobilizing agents. These results demonstrated that PKC does not prevent the initiation of the apoptotic signal, which occurs within the first 12 hour, but it blocks the downstream events of Ca^{+2} triggered apoptosis[14].

In this study we have found that the increased apoptosis in group treated with levamisole is accompanied by decreased Ca ATPase. The probable action of levamisole as inducer of apoptosis is through inhibition of the activity of Ca^{+2} ATPase which lead to increase intracellular Ca^{+2}. The increased intracellular Ca leads to activation of Ca^{+2} dependent nuclease which cause specific DNA cleavage and hence the activation of apoptotic process[3]. Levamisole is a stimulator of C-GMP and hence increase intracellular Ca which led to apoptosis[15]. Also levamisole can increase the expression of bax protein which is enhancer of apoptosis and can decrease the expression of bcl-2 which inhibits apoptosis[16,17]. Decreased activity of Ca ATPase *per say* probably due to inhibition of ATP by levamisole, Levamisole is protein kinase C inhibitor. PKC which is a stimulator of protein synthesis so inhibition of PKC lead to apoptosis[18], the action of Levamisole through inhibition of PKC and c.AMP dependant protein kinases will ameliorate Ca release from internal store, so will further inhibit Ca^{+2} ATPase activity in group treated with levamisole[19].

Conclusion

levamisole induces apoptosis in rat thymocyte by decreasing Ca ATPase activity, The exquisite sensitivity of thymocyte and hence lymphocytes to calcium-dependent apoptosis in the presence of PKC inhibitors like levamisole could provide the basis for new treatment programs against many diseases such as leukemia, lymphomas, or autoimmune diseases.

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EFFECT OF MACROMOLECULES ON ERYTHROCYTES AGGREGATION AND SEDIMENTATION KINETICS BY HE-NE LASER SCATTERING

R. Al-Khazragi[1] *B.Sc. M.Sc.*, **T. Al-Wajidi**[2] *B.Sc. Ph.D.*, **Fakher S. Al-Ani**[1] *M.Sc. Ph.D.*

Abstract

Background: The erythrocyte aggregation is an important physiological phenomenon in the circulation of blood. It is a basic characteristic of normal blood that plays a major role in the cardiovascular system, especialy in the microcirculation.

Objective: To evaluate the role of macromolecules of red cell suspending medium on the aggregation and sedimentation of red blood cells.

Subjects & Method: The present syudy was done on forty one healthy subjects. Laser light is passed through a well mixed sample of blood. From scattered light intensity profiles continuously obtained during aggregation of erythrocytes and sedimentation of the aggregates, the intervals of rouleaux formation one- dimensional aggregate and three- dimensional aggregate formation were computed.

Results: The study showed that the time neended for the stages of aggregation and sedimentation of RBCs suspended in dextran 500,000 mol.wt is significantly less ($p < 0.001$) than thesame stages of RBCs suspended in normal plasma. The fibrinogen neended for rouleaux formation while Immuno-globulen neended for one and three-dimensional aggregate formation.

Conclusion: We observed that the availability of macromolecules and proteins and their characteristics (mol. Wt. and configuration) have a critical role on each stage of aggregation process.

Key words: Erythrocyte aggregation, sedimentation rate, macromolecules, laser light.

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Introduction

Erythrocytes aggregation and disaggregation are natural phenomena in the circulation of blood[1]. Aggregation of red blood cells is the formation of reversible structure containing a number of particles, while erythrocytes sedimentation monitor the tendency of unstirred red blood cells to form aggregates in plasma[2].

The process of aggregation affected by many of physical and chemical properties such as hematocrite, Ph, of the suspending medium, macromolecules and flow conditions[3,4].

The macromolecules are essential for the aggregation of erythrocytes in bridging among erythrocytes. Red cell aggregation is induced by shear rate-dependent and reversible bridging of plasma proteins high molecular weight at their both ends between surfaces of two adjacent red cells[5,6].

Materials & Methods

The present study has been depended on a method that modified from a method to E. Muralidharan, in Biorheology, 1994[7] but work on the same principles, that is using laser light scattering.

Fresh blood samples were obtained from the cubital vein of 41 healthy human subjects with heparin (0.03/5ml of blood). Samples were centrifuged at 3000 rpm for 10 min at room temperature. Plasma was separated from the red blood cells and divided into two parts. Control

[1]Dept. Physiology (College of Medicine, Al-Nahrain University) [2]Dept. Physiology (Al-Mustansiriya University)

Address correspondence to Miss. R. Al-Khazragi.

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erythrocytes were obtained by washing three times with isotonic phosphate buffered saline solution (50mM Sodium Phosphate, 3mM KCl, 90mM NaCl, 0.1g/dl D-glucose, PH 7.4).

Re-centrifugation for one part of the separated plasma with high speed centrifuge at 4°C and at rate of 15000 rpm to get macromolecules free plasma then adding bovine albumin of 0.5g/dl, to prevent adhesion between RBCs and the wall of chamber, to prepare sample of 10%PCV value and compare the results with that of plasma full of macromolecules.

The system of the measurement is shown in Figure 1. A linear polarized He-Ne laser source of wave length (632.nm), generation power (1mW) and beam diameter (1mm) (Griffin Co.) was passed through erythrocyte suspension in a chamber (50×10×1)mm made of a microscopic glass plates. Blood column height was kept at 40mm. The foreword scattered light intensity through the sample column was detected with a photocell (photodiode amplifier).

The photocell placed in front of the laser beam and allowed the beam to pass directly through the crystal of the cell. The signals from the photocell are passed through a light flexible cable to an amplifier (Grass 7P1F) for signal amplification. The sample chamber was mounted firmly on the holder so that the laser beam passed, exactly, through the center of the chamber.

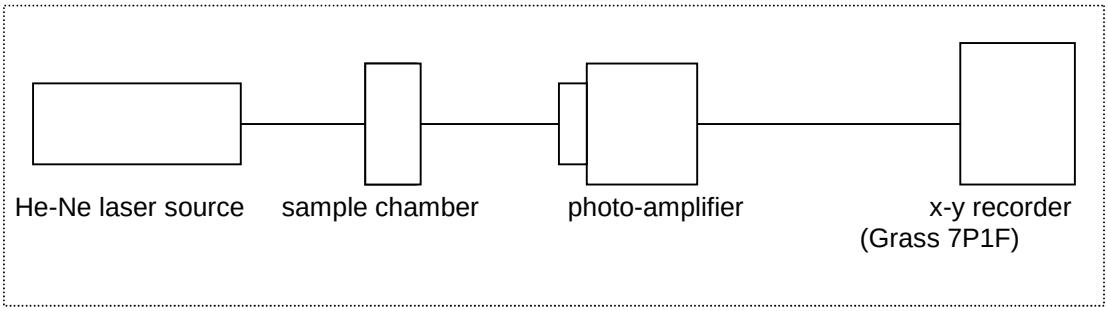


Figure 1: The system layout

The blood sample was gently introduced into the chamber by using a syringe with long needle. Immediately after the sample was introduced, the forward-light signal was continuously recorded by the system.

Results

Figure 2 shows the pattern of rouleaux formation, one-dimensional aggregate and three- dimensional aggregate formation curve, of sample with 10%PCV suspended in plasma as it recorded by laser assisted aggregometer used in this study.

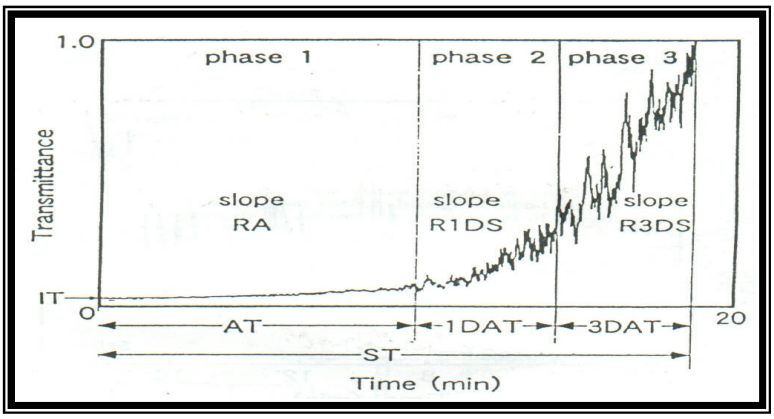


Figure 2: Pattern of different stages of aggregation and sedimentation as recorded by laser scattering techniques.

There was a slight increase in the signal due to the reorientation of single erythrocytes when the erythrocytes were mono dispersed in the beginning of the aggregation process. The sedimentation of the aggregates formed was indicated by the appearance of fluctuations in the signal. These fluctuations were smaller in the beginning and became larger towards the end. The time at which the first sharp fluctuation appeared in the signal was termed AT (aggregation time). These fluctuations continued until the signal reached the maximum without any variation. The time at which the signal reached the maximum was termed ST (sedimentation time).

The initial phase was due to the movement of single erythrocytes in the process of forming small aggregates. The rate of aggregation (RA) was obtained from the slope of this phase. The second phase was due to the sedimentation of small and one-dimensional aggregates. The duration of this phase was termed 1DAT (one-dimensional aggregation time). The slope of this phase provided the rate of sedimentation of one-dimensional aggregates (R1DS). The third phase was due to the sedimentation of large and three- dimensional aggregates. The duration of this phase was termed 3DAT (three-dimensional aggregation time). The rate of sedimentation of the three-dimensional aggregates (R3DS) was obtained from the slope of this phase. The light intensity fluctuation showed a clear visible in the signal between these phases.

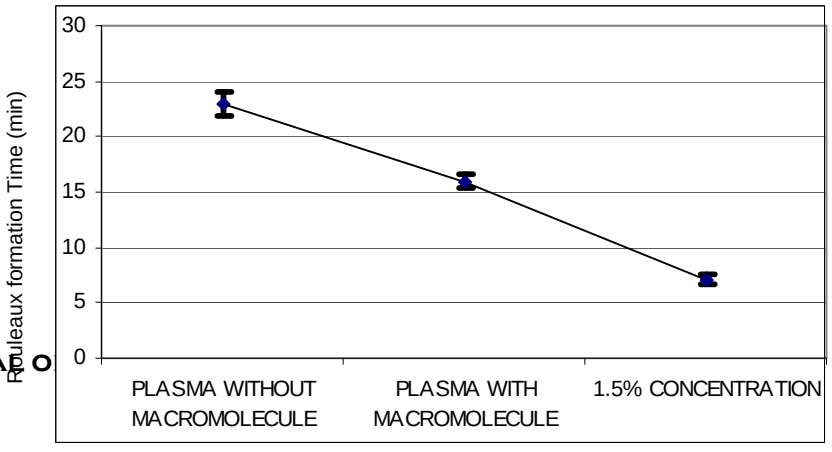
The time needed for the stages of aggregation and sedimentation of RBCs suspended in dextran (500.000) (1.5% concentration solution) is significantly less ($P < 0.001$) than the time require for the stages of aggregation and sedimentation of RBCs suspended in normal plasma (contain proteins and platelets) of the same viscosity Table 1:

Table 1: Effect of macromolecules on aggregation and sedimentation time

1.5% Dextran concentration	No.	Plasma with macromolecules	Plasma without macromolecules	Parameters
AT (min)	41	22.92±1.1395	15.94±0.645*	7.112±0.515***
1DAT (min)	41	14.344±1.327	4.98±0.324*	3.63±0.374*
3DAT (min)	41	23.4±1.853	9.47±0.753*	9.45±0.888*
ST (min)	41	60.68±3.7654	30.63±1.423*	20.17±1.59***

* comparison with plasma without macromolecules, ** comparison with plasma with macromolecules

From the same table we can show that the time needed for the stages of aggregation and sedimentation of RBCs suspended in normal plasma is significantly lower ($P < 0.001$) than the time needed for the stages of aggregation and sedimentation of RBCs suspended in plasma free of macromolecules (after centrifugation with high speed centrifuge) Figures 3 & 4.



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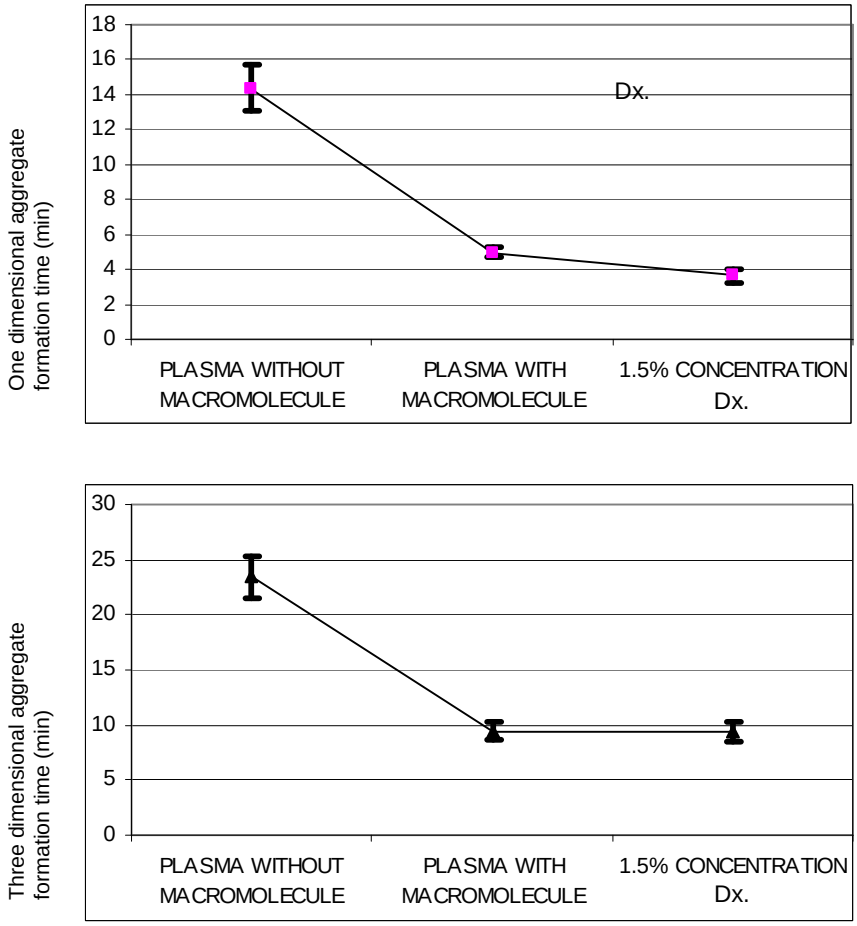

Figure 3: Effect of macromolecules on the time of aggregation stages

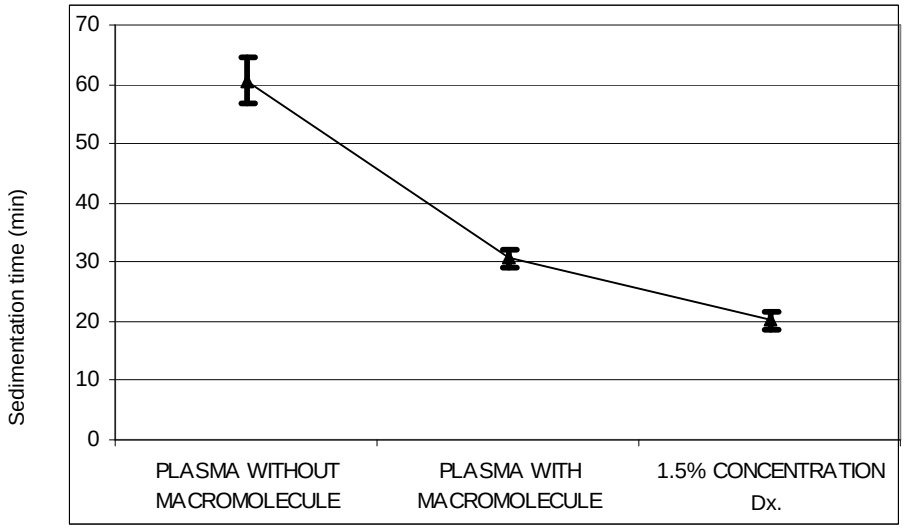

Figure 4: Effect of macromolecules on sedimentation time

Discussion

From this study, it is found that the presence of macromolecules in suspending medium of red blood cells play a major role in aggregation process. It had been found that the time needed

for rouleaux formation and one dimensional aggregate for red blood cells suspended in macromolecules free plasma is significantly greater than that needed for rouleaux formation and one-dimensional aggregate for red blood cells suspended in macromolecules full plasma[8-10].

Due to that the aggregation of red blood cells depends mainly on the availability of the macromolecules in normal suspending medium (plasma) such as fibrinogen, Immunoglobulin[10].

All these macromolecules act as an attractive force (bridging), between the two adjacent erythrocytes because these macromolecules have a positive charge so it contact with red blood cells due to its negative surface charge and thus reduce the repulsive force (the electrostatic repulsive energy (E_e) and induces the binding energy (E_b) and that lead to increases the net aggregation energy eq.

$$E_a = E_b - E_e - E_m - E_s \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where E_a = the net aggregation energy, E_b = the macromolecules binding energy, E_e = the electrostatic repulsive energy, E_m = the cell deformation energy during aggregation, Es = the mechanical shear energy. In the absence of the mechanical shear energy Es=0[11-12].

For this reason red blood cells alone in salt solution do not adhere to each other even if ionic strength is increased or if charged groups on the cell surface are modified[13].

The ability of plasma proteins to promote adhesion and aggregation of red blood cells is a significant factor in blood rheology.

From the same figure, we can found that the time needed for rouleaux formation and one-dimensional aggregate for red blood cells suspended in high mol. Wt. dextran solution is significantly less than the time needed for rouleaux formation and one-dimensional aggregate for red blood cells suspended in macromolecules full plasma which have the same viscosity (the same concentration of dextran and plasma proteins) this result is due to increasing the concentration and the molecular weight of dextran which accelerate the erythrocyte aggregation and also the molecular configuration affect erythrocyte aggregation[5, 8,10].

Because the viscosity (concentration) of dextran solution and the macromolecules full plasma with the same viscosity so the result of the decreasing time related to the high mol. Wt of dextran (500,000) which is greater than that of fibrinogen which has mol. Wt (340,000) higher than any other mol. Wt of plasma proteins. In addition, this result is due to increasing the molecular length of dextran (500,000) is (1580°A) which is longer than that of fibrinogen (477°A), the molecular length of Dx. And fibrinogen induces both rouleax formation and one- dimensional aggregate rate[14,15].

Dextran induces erythrocyte aggregation by macromolecular adsorption on the membrane and connecting adjacent erythrocytes by bridging.

The tendency of cells to aggregate ranges from very weak, as observed in rouleaux formation, to very strong, as seen for cells in the presence of different concentrations of high molecular weight dextran[13].

Morphologically, with increase in molecular weight of dextran the size of erythrocyte aggregates increased and their structure became tightly packed[6].

On the other hand, it can be shown that the time needed for three dimensional aggregate for red blood cells in dextran solution is slightly (insignificantly) less (or almost equal) to the time need for three dimensional aggregate for red blood cells suspended in macromolecules full plasma of the same viscosity, this may be related to the higher effect of proteins (globulin, fibrenogim) on the three dimensional aggregate formation according to there molecular structure.

Their role in increasing three dimensional aggregate of erythrocyte is due to its configuration (globular-like shape) where the rate of rouleaux formation increased proportionally to fibrinogen concentration which seemed to be less effective for the

mation of (3DA) than γ-globulin, while (3DA) formed very quickly due to the presence of γ-globulins[9,14].

Removing or changing the macromolecules of the suspending medium of RBCs will not affect the sedimentation rate as the rouleaux formation, one and three dimensional aggregate formation rate. The variability in the sedimentation rate depends on the variability of aggregation rate so that the higher aggregation rate the higher sedimentation rate.

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THE USE OF CHROM AGAR ORIENTATION FOR THE DETECTION OF UROPATHOGENS

Manal A. Habib[1] *M.Sc. Ph.D,* **Elham Al-Kaisi**[2] *Ph.D.*, **Layla S. Al-Omar**[1] *B.Sc. Ph.D.*

Abstract

Background: CHROM agar Orientation is a chromogenic medium used for detection and differentiation of bacterial growth in clinical samples.

Objectives: Evaluation of CHROM agar Orientation for identification of urinary pathogens in comparison to the ordinary media used.

Methods: CHROM agar Orientation, blood agar and MacConkey agar media were used for direct inoculation of 415 urine samples from patients with urinary tract infection (UTI).

Results: CHROM agar Orientation succeeded in detecting all the urine pathogens that were detected by the reference media including Gram negative bacilli, Gram positive cocci and yeast but further biochemical tests were needed to differentiate the uropathogens to species level.

Conclusion: CHROM agar Orientation medium enabled excellent detection and presumptive identification of urinary pathogens both in pure and mixed culture, therefore, can replace the standard primary plating media used in routine diagnosis of urinary tract infection.

Key words: CHROM agar Orientation, urine.

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Introduction

Urinary tract infection is one of the most common diseases. It is responsible for a significant share of the workload in many clinical microbiology laboratories[1-3]. Rapid identification of the infecting organism provides useful information to the clinician for appropriate antibiotic choice prior to organism susceptibilities being available[4,5]. In the last few years several chromogenic media have been developed and commercialized allowing for more specific direct differentiation of microorganism on primary plates[6,7]. CHROM agar orientation, offers simultaneous presumptive identification of Gram positive and Gram negative genera of bacteria and yeast on a single medium by means of distinct colony colors produced by reactions of genus or species specific enzymes with a suitable chromogenic substrate incorporated into the agar[5]. So the main objective of this study was to evaluate UTI diagnostic performance of CHROM agar Orientation medium and to compare this performance to that of the reference media (blood agar and MacConkey agar).

Materials & Methods

Study population & specimens:

A total of 415 midstream urine samples collected from outpatients and hospitalized patients (from Al-Khadhimiya Teaching Hospital), having signs and symptoms of urinary tract infection (UTI) like dysuria, frequency of micturition, fever and lower abdominal pain, were tested in this study which were carried out from Dec. 2001 to Jan. 2002.

Media preparation:

CHROM agar Orientation was prepared according to the manufacturers instructions (CHROM agar Company, France), where 32.4 gm of CHROM agar Orientation powder were dissolved in one Liter of double distilled water with slow

[1] **Dept. Medical Microbiology, College of Medicine, Al-Nahrain University, Iraq,** [2] **Al-Petra University/ Faculty of pharmacy and Medical Techniques, Jordan.**

Address correspondence to Dr. Manal A. Habib, Email: manalviro@yahoo.com

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rotation, then the medium was boiled under continuous stirring then autoclaved for 15 min. The medium then cooled to 45ºC and then dispensed in sterile petri dishes and allowed to solidify at room temperature. The plates then were stored at 4ºC in a dark container and used within one month.

Methods

After inoculation of the specimens on blood, MacConkey and Chromagar media, the plates were incubated at 37ºC for 16-24 hours in the dark[5]. All isolates from members of the family of Enterobacteriaceae were identified using the API reference profile system (Biomerieux, France), with tests performance as instructed by the manufacturer.

Streptococci were confirmed by hemolysis on blood agar and Gram stain. *S. aureus* was Gram stained and checked for coagulase test and manitol fermentation.

Candida isolates were identified by sugar fermentation and by testing for germ tube formation. Other tests like oxidease and catalase utilization were performed by following the instruction of the manufacturer.

Control strains of gram positive and gram negative bacteria were obtained from Medical Microbiology Department, College of Medicine, Al-Nahrain University. They were used as quality controls for the media and to assess color stability. A growth of more than 10 colony-forming units/ml was considered as a significant growth of bacteria.

Results

Out of 415 urine samples tested, only 178 specimens revealed growth as a single (91.6%) or mixed (8.4%) cultures. The results have also shown that 66.8% of the UTI were caused by Gram negative bacteria, 25.4% by Gram positive bacteria and only 7.8% by *Candida spp.* (Figure 1).

E coli (n=52 isolates) was found to be the predominant isolates (26.9%) from positive urine samples. Of the *E. coli* isolates tested, all but 2, produced red to pink colonies.

Enterobacter spp., *Klebsiella spp.* and *Citrobacter spp.* demonstrated blue colonies. However, one isolate of *Citrobacter spp.* revealed colorless colonies. *Pseudomonas spp.* (n=13) produced translucent creamy colonies.

Proteus spp. (n=5) resulted in characteristic clear colonies with brown halo. Only two isolates of *Serratia* were obtained and both appeared as blue colonies. *S. aureus* isolates (n=8) gave small golden colonies except 2 isolates that gave white opaque colonies.

S. epidermidis. (n=26), *Streptococci* (n=4) and *Enterococci spp.* (n=11) appeared as small creamy, small turquoise and diffuse blue colonies respectively. Finally 15 isolates of *candida* species were all creamy in color (Table-1).

All mixed cultures were identified in CHROM agar Orientation medium while only 66.6% and 20% of these cultures were detected on blood and MacConkey agar respectively (Table-2, figure 2).

All control and test Gram negative organisms grew equally well on CHROM agar Orientation and on MacConkey agar. This similarity of colors produced by *Enterobacter spp., Citrobacter spp*. and *klebsiella spp*. prevents differentiation among them and additional biochemical tests were required for their final identification.

In the dark, colors of the colonies were stable. Moreover, when exposed to light the color changed while longer incubation in the dark made the colors deeper making identification easier.

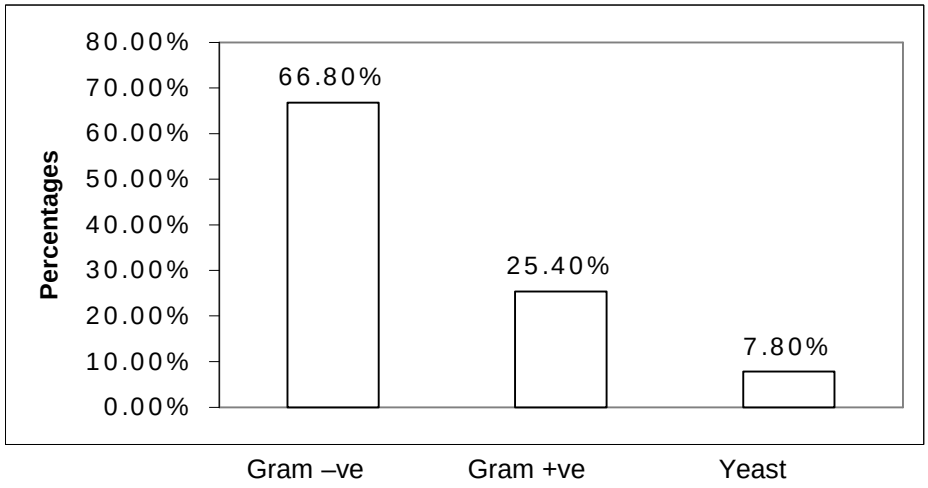


Figure 1: The percentages of Gram -ve, Gram +ve and yeast in positive urine samples

Table 1: The color of colonies on CHROM agar Orientation

General	No. of isolates (%)	No. of isolates with described color (%)	Description of color &colony morphology
E. coli	52 (26.9)	50 (96.2)	Red to pink
		2(3.8)	Colorless
Enterobacter spp.	26 (13.5)	23 (88.5)	Metalic blue
		3 (11.5)	Light blue
Klebsiella spp.	22 (11.4)	22 (100)	Metalic blue
S. epidermidis.	26 (13.5)	26 (100)	Creamy, small
Candida spp.	15(7.8)	15 (100)	White creamy, convex
Citrobacter spp.	9 (4.7)	8 (88.9)	Metalic blue
		1 (11.1)	Colorless
Pseudomonus spp.	13 (6.7)	13 (100)	Translucent, creamy
S. aureus	8 (4.1)	6 (75)	Golden, opaque, small
		2 (25)	White
Proteus spp.	5 (2.6)	5 (100)	Colorless with brown halo
Enterococci spp.	11 (5.7)	11 (100)	Diffuse blue
Serratia spp.	2 (1)	2 (100)	Light blue
Streptococci spp.	4 (2.1)	4 (100)	Turquoise, small
Total	193	193	

Table 2: Distribution of isolates from positive urine samples on CHROM agar, Blood agar and MacConkey agar

	CHROMagar		Blood agar		MacConkey agar	
Genera of bacteria	**Total No. of isolates (%)**	**Pure culture (%)**	**Total No. of isolates (%)**	**Pure culture (%)**	**Total No. of isolates (%)**	**Pure culture (%)**
E. coli	52 (26.9)	3(82.6)	50(26.5)	43(86)	52(36.6)	3(82.6)
Enterobacter spp.	26 (13.5)	26(100)	26(13.8)	26(100)	26(18.3)	26(100)
Klebsiella spp.	22 (11.4)	21(95.4)	22(15.4)	21(95.4)	22(15.4)	21(95.4)
S. epidermidis	26 (13.5)	15(57.6)	26(13.8)	15(57.6)		
Candida spp.	15(7.8)	9(60)	12(8.4)	9(75)	13(9.1)	7(53.8)
Citrobacter spp.	9 (4.7)	9(100)	9(100)	9(100)	9(6.3)	9(100)
Pseudomonus spp.	13 (6.7)	12(92.3)	13(6.9)	12(92.3)	13(9.1)	12(92.3)
S. aureus	8 (4.1)	7(87.5)	8(4.2)	7(87.5)		
Proteus spp.	5 (2.6)	5(100)	5(2.6)	5(100)		
Enterococci spp.	11 (5.7)	10(90.9)	11(7.7)	10(90.9)	5(3.5)	5(100)
Serratia spp.	2 (1)	2(100)	2(1.0)	2(100)		
Streptococci spp.	4 (2.1)	4(100)	4(2.1)	4(100)	2(1.4)	2(100)
Total	193	163	188	163	142	125

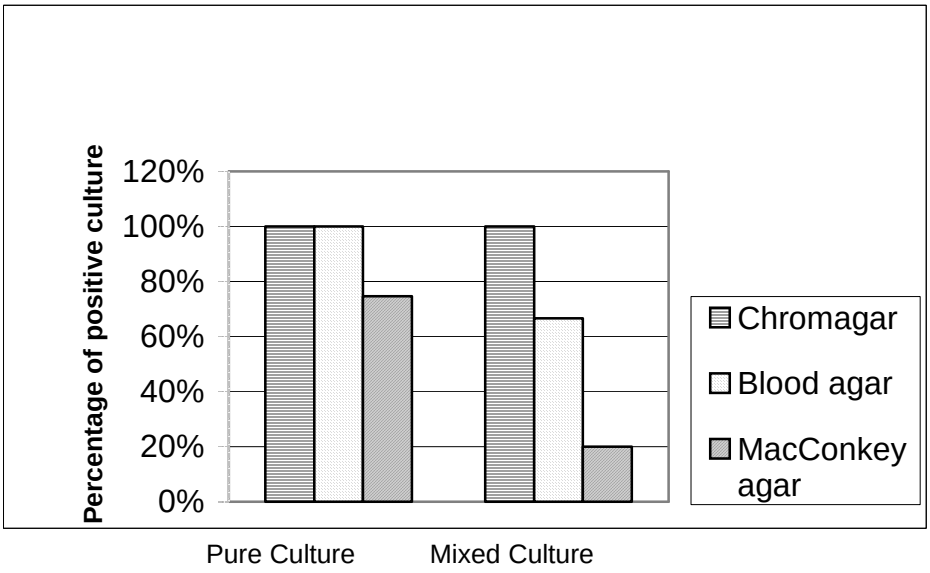
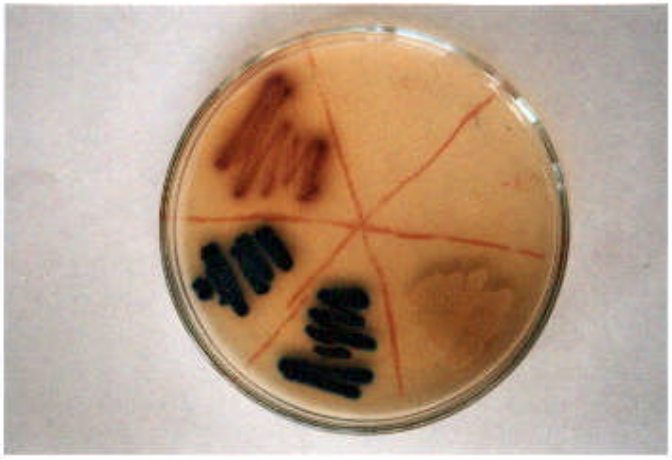


Figure 2: Ability of different media to isolate microorganism in positive urine samples



Picture 1: Color reaction of microorganism on CHROMagar Orientation
1: *Psudomonas spp.* 2: *Klebsiella spp.* 3: *Enterobacter spp.* 4: *E. coli*

Discussion

The present study aims to evaluate, for the first time in Iraq, CHROM agar Orientation medium as a direct isolating medium for Gram positive, Gram negative bacteria and yeast from urine specimens. A total of 415 urine samples were tested by parallel inoculation on CHROM agar orientation and on two reference media (blood and MacConkey agar). CHROM agar Orientation showed the same ability to detect urine pathogens as the combination of the two reference media used making it an attractive primary screening medium for differentiation and presumptive identification of bacterial genera and species on the bases of colony color and morphology. Several studies have reported similar conclusion[5,7,8].

This study also shown that 66.8% of UTI was caused by gram negative bacteria considering that the majority were *E. coli* and that this microbe is responsible for the most nosocomial and community acquired UTI[9,10]. All isolates of *E.coli*, but two, grew on CHROM agar orientation in reddish to pink colonies and were easy to be distinguished. The remaining two isolates appeared as colorless colonies and could be easily differentiated by additional biochemical tests. These results correlate with findings observed by other workers[5,7]. The medium failed to differentiate *Klebsiella spp., Enterobacter spp.* and C*itrobacter spp.* owing to similarity of color produced and

final identification among them require other biochemical tests[5].

Since 1905 the most widely used medium in the clinical laboratory for the isolation and differentiation of coli form bacteria and enteric pathogens has been the MacConkey agar[11]. MacConkey agar differentiates Gram negative bacteria by determining lactose utilization with a neutral red indicator. Red or pink to colorless colonies are produced depending on the ability of the isolate to ferment lactose. Careful observers are often able to recognize mixtures of gram negative bacteria on a single plate, but the absence of any differential genus-specific indicator property in the MacConkey agar means that there is no guarantee that mixed coliform cultures are always detected. CHROM agar Orientation permit good discrimination of species of bacteria in mixed culture after direct plating of urine specimen, based on color produced by reaction of genus or species specific enzymes with appropriately chromogenic substrate. CHROM agar Orientation also offered the advantages of limiting the spread of some isolates of bacteria such as *Proteus spp*. and *Klebsiella pneumonae* and some strains of *E. coli* which may yield confluent growth on plates[5,7,8,12]. This will enhance the ability of the medium to detect urinary pathogens when mixed flora is present[5]. The colors of the colonies on CHROM agar Orientation were found to be stable in the dark, but changed color were observed when plates exposed to light for a period of time. This means that plates should be taken out of the dark environment just prior to initial reading. Light sensitivity is one of the disadvantages when using this medium. Another disadvantage is that laboratory staff with color blindness will have difficulty in distinguishing differences in colors of the colonies. However this medium allowed a remarkable reduction in the workload and a significant saving of time. On the basis of their preference these media can replace the standard primary plating media used in routine diagnosis of UTI.

In conclusion CHROM agar Orientation medium represent an attractive and excellent screening medium for reliable detection and presumptive identification of UTI pathogens especially when there is a mixed culture. Moreover, the use of this medium for other clinical specimens requires further investigations.

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A COMPARISON BETWEEN TWO ANTIGENS OF CANDIDA ALBICANS FOR THE DIAGNOSIS OF SYSTEMIC CANDIDIASIS

Azhar A.F. Al-Attraqchi *M.Sc., Ph.D*

Abstract

Background: In most instances clinical and mycological features alone do not provide sufficient evidence for confident diagnosis, so that information is usually required before an equivocal is obtained for systemic antifungal treatment.
Systemic candidiasis is a disease of immunocompromised individuals. Many techniques are used as serodiagnostic tools, but almost all of them gave disappointing results. One of the most specific antigens, for candida albicans may be used for such purpose is cytoplasmic antigen.

Objectives: To compare two types of antigens of C.albicans and used for serodiagnosis of systemic candidiasis.

Materials & Methods: Standard strain of Candida albicans (ATCC 10231) cultured on Sabouraud broth employed in this study supplied from Biotechnology and Molecular Biology Department/ Baghdad University. Blood samples are collected from (100) patients with acute lymphoid and myloid leukemia admitted different Iraqi hospitals. Sputum or Oral Swabs were taken from those patients. Blood Samples were cultivated in Brain heart infusion broth. -Phosphate buffer saline was 7.0 and agarose concentration was 1.5%.

Results: Eight patients out of (100) developed systemic candidiasis according to precipitation reaction by using cytoplasmic antigens, while (77) out of those (100) gave positive precipitation reaction were cell wall antigen was used.

Conclusions: Cytoplasmic antigen is a suitable antigen to be used as a serodiagnosis of systemic candidiasis .It gave only 8% of results, while the cell wall antigen gave 77% of the total number of patients examined.

Key words: Candida albicans , systemic candidiasis

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Introduction

Systemic candidiasis is a disease of immunocompromised individuals. It is a rare disease and usually occurs in patients with malignancies[7] or recipients of immuno suppressive drugs[12].
Candida infection may be a secondary invader of different organs where a preexisting disease is present. Clinical and mycological features alone do not provide sufficient evidence for diagnosis, so serological evidence is commonly an important adjunct to the diagnosis of this infectious disease[4].
The incidence of systemic candidiasis increased alarmingly later on[5]. These infections demand an accurate differential diagnosis, because specific therapy with amphotericin B initiates the risk of toxic side effects. Early diagnosis and therapy, essential for a favorable prognosis are impeded by the lack of pathognomonic clinical signs. Diagnosis rests entirely upon isolation of Canadida from blood and other internal fluids and organs[8]. Such cultures may require two or more weeks of incubation before candidal growth appears. In renal candidiasis, the blood cultures are frequently negative[6,10]. Therefore, there is a need for a good diagnostic tool for systemic candidiasis.
There are different antigens that may be used for such purpose such as cell wall antigens which are considered as major determinants of the antigenic structures of different species[11], culture filtrate antigens, antigens specific to C. albicans hyphae, or broken-cell extracts (cytoplasmic extracts).

Material & Methods

Candida albicans standard strain (ATCC 10231) was obtained from the Biotechnology and Molecular Biology department-Baghdad University, cultured

Dept. Medical Microbiology, College of Medicine, Al-Nahrain University.
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on Sabouraud broth. Blood samples, as 10 ml each, were collected from 100 patients with acute lymphoid or myloid leukemia. Five ml of each sample was used for serum preparation and the other 5 ml used for blood culture.

Similarly 10 ml of blood were collected from each of four healthy individuals as a control for the same purposes. Brain heart infusion was added to blood culture media of which 5ml was used for the isolation of Candida albicans. Agarose of a concentration of 1.5% was used for precipitation reaction. Sputum or oral swabs were taken from those patients for culture purposes.

Cell Wall preparation of C. albicans

Cell wall of C. albicans was prepared according to (Chaffin and Stocco, 1983)[3] with modification *:

Five ml inoculum of standard strain (ATCC 10231) in Sabouraud broth was reinoculated into 300 ml of Sabouraud broth in a 500 ml Erylenmyr's flask and incubated in a shaker incubator for 24 hours. Cultures were harvested at its logarithmic phase, and then centrifuged at 1000 x g for 15 min. An equal volume of crushed glass was added to the pelette and blended in a vortex mixer for (3) hours with intervals in ice*.

As determined by light microscopy cell breakage was as high as 90%. The crushed glass was repeatedly washed with ice-cold distilled water until the supernatants were clear. The supernatants were combined and the cell walls harvested by centrifugation at 3000 x g for 10 minutes.

The pelette was washed 10 times in ice-cold distilled water as described by Cassone et al., (1979)[9]. The cell wall proteins were solubilized by boiling for 2 minutes in sample buffer 2 x SDS 100ml (tris base 1.52 gm; glycerol 20 ml; SDS 2.0gm; 2-mercaptoethanol 2.0 ml; Bromopherol blue 1mg) pH 6.8, according to laemli (1970)[9]. The amount of protein was determined by Biuret method[13].

Extraction of Cytoplasmic Protein

This procedure was followed according to Taschdjion et al., 1966[14] and the procedure of Chaffin and Stocco (1983)[3] with modifications.

A standard strain (ATCC 10231) of C. albicans was inoculated in 100 ml sabouraud broth in 250 ml. Erlenmeyer flask, incubated at 37°C for 10 hours in a shaker incubator*(to obtain the logarithmic phase of C.albicans)[1].

The number of yeast cells was determined with a hemocytometer chamber and $1.3x10^5$ cell/ml was prepared. Two rabbits weighing 3 Kg were inoculated with $1.3x10^5$ cells/mli.v.

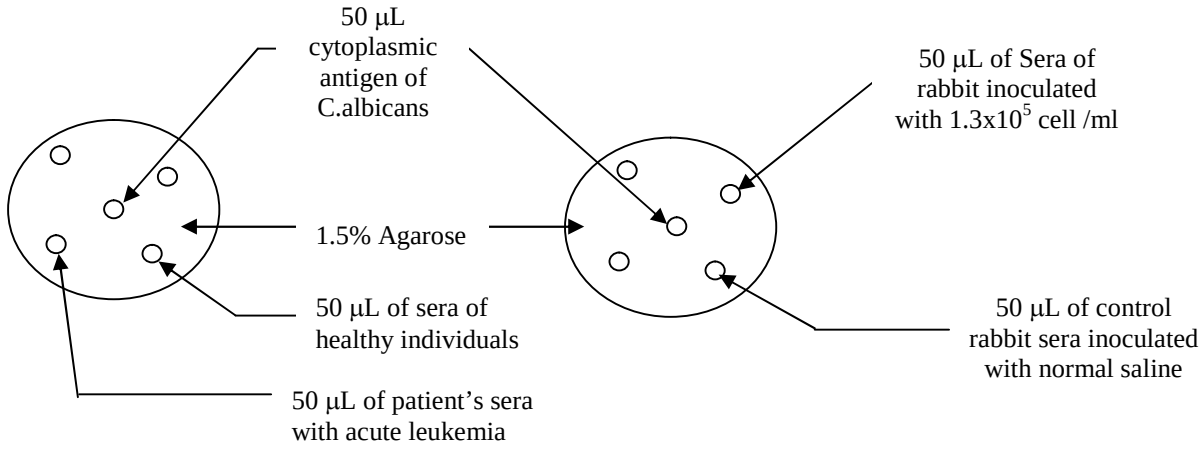
Another 2 rabbits of the same weight were inoculated with 1 ml normal saline. In order to prepare a particular antigen to be used for determination of systemic candidiasis, a cytoplasmic antigen was prepared for such purpose. One liter of sabouraud broth was inoculated with 10 ml of C. albicans (ATCC 10231) at the logarithmic phase.

The culture was incubated in a shaker incubator at 37°C for 10 hours*. The pellet was prepared by centrifugation at 1500 x g for 15 minutes. An equal volume of crushed glass was added to the pellet and the mixture blended in a vortex mixer of a total of 3 hours with intervals in ice*. As determined by light microscopy cell breakage was as high as 90%.

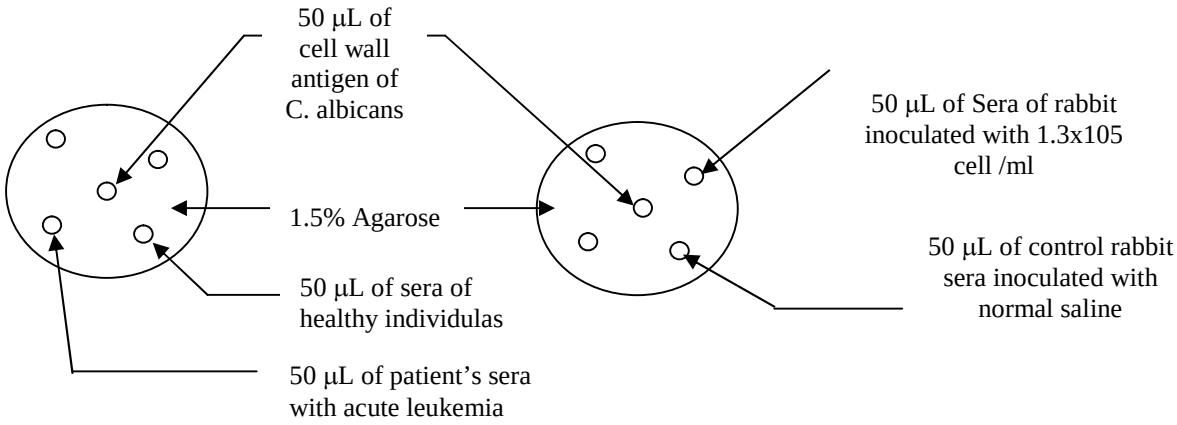
The supernatant was used. The pellet was washed with iced cold distilled water repeatedly until the supernatant was clear. The supernatants were combined and centrifuged at 1500 x g for 15 minutes. The supernatant was kept at -20°C overnight and then recenterifuged at the same conditions.

The final supernatants representing cytoplasmic antigen which was grayish and opalescent in color and was cell-free on microscopic examination. The solution was concentrated in a dialysis tube against sucrose and used as cytoplasmic antigen.*. The antigen was used in the precipitation test for detection of systemic candidiasis.

The sera were collected from previously inoculated rabbits with $1.3x10^5$ cells/ml and of those inoculated with normal saline. Agarose of a concentration of 1.5% was poured in Petri dishes. They were left to dry then punched with corck porrer as explained in the following figure:



A diagram explains the procedure of inoculation of C. albicans cytoplasmic antigen against sera of rabbit and patients with leukemia



A diagram explains the procedure of inoculation of C. albicans cell wall antigen against sera of rabbit and Patients with leukemia

Fifty µL of C. albicans cytoplasmic antigen and cell wall antigen was inoculated in the middle well of each plate separately surround by the sera of rabbits, and of patients. Plates were incubated in a humid chamber at 37°C for 48-72 hours. Precipitin lines were detected then.

Results

With cytoplasmic antigen a precipitation reaction was seen in only 8 patients, while with cell wall antigen 77 such reaction were seen. Sera of four healthy individuals showed no reaction with cytoplasmic antigen, while they gave positive results with cell wall antigen. Blood culture showed only 2 positive growths of C. albicans.

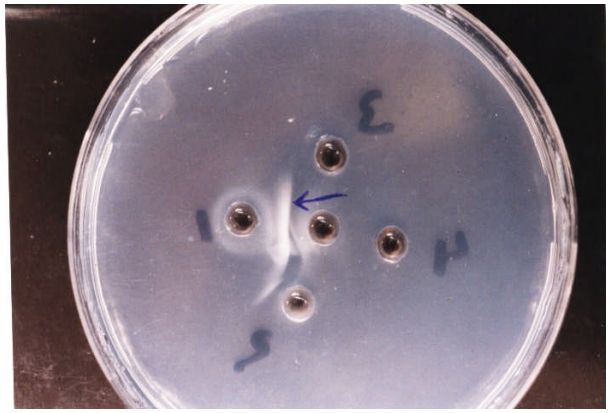


Figure 1: precipitation reaction between rabbit sera inoculated with 1.3 x 10^5 line *C.albicanc* cell/ml and cytoplasmic antigen . A clear precipitation line occurred between the sera and the antigen, marked with arrow pointer

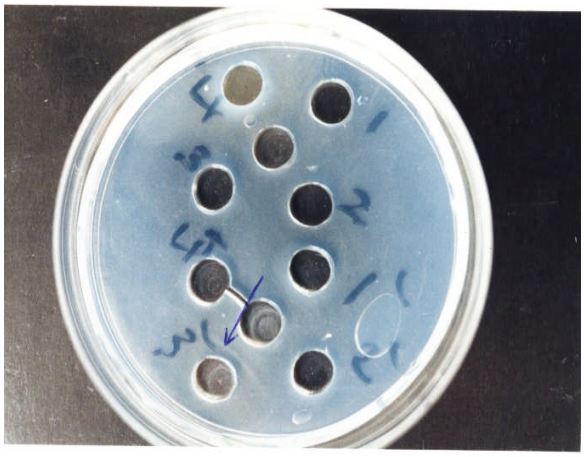


Figure 2: Shows precipitation reaction between sera of leukemic patients and *C. albicans* cytoplasmic antigens, marked line representing precipitation line

Discussion

As determined by blood 2% only were positive as candidemia (systemic candidiasis), while by serological tests the results were different. When we used the cell wall of broken cells of C. albicans was used as an antigen against the sera of leukemic patients, positive reaction occurred with 77% of those patients sera, while only 8% of this reaction occurred with the sera of the same patient's when cytoplasmic antigen of C. albicans was used .The sera of healthy individuals gave precipitin lines with the cell walls of C. albicans, while they gave no such lines against cytoplasmic antigen. From that we can conclude that the cell wall of C. albicans cannot be used as a serodiagnostic tool for systemic candidiasis in contrary to cytoplasmic antigen. To explain this phenomenons: C. albicans is carried as normal flora in the gastrointestinal tract or mouth cavity in healthy individuals ,therefore cell wall antigen usually faces the immune system of those carriers. When the disease occurred by this organism the antibodies of those individuals triggered against the first antigen facing them, representing in the cell wall antigen, the antibodies responsible for precipitation reaction are the same in both healthy individuals and patients that gave positive reaction. Cytoplasmic antigen which is the almost inner antigen and triggered only in the case of systemic candidiasis and the immune system responsible for damaging Candida cells cells release their contents including cytoplasmic antigen which is not present in healthy individuals. So cytoplasmic antigen could be used as a serodiagnostic tool for systemic candidiasis.

As mentioned previously only 8% of systemic candidiasis was found by using cytoplasmic antigen and this disagrees with Taschdjian et al., 1988[14] who found that 85% of different groups of patients had developed systemic candidiasis and detected by using cytoplasmic antigen. This may be explained on the following probabilities: First; the variability in the virulence of strains included in both studies, Second; the variability in the patient groups included in both studies, patients in this study were suffering from acute leukemia, while they included different groups of patients namely leukemic, diabetic, rheumatic heart diseases, narcotic addicts, and those patients receiving intravenous fluids, antibiotics and steroids. It is important to notice that nowadays the use of amphotericin B and chemotherapy are more widely used than previous time. Most of patients in this study were receiving these drugs, and this may be an important factor in reducing the number of systemic candidiasis.

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PROGNOSTIC EVALUATION OF INITIAL BONE MARROW HISTOPATHOLOGICAL FEATURES IN CHRONIC MYELOGENOUS LEUKEMIA

Saad S. Mansoor[1] *FRCPath*., **Subh S. Al-Mudalal**[1] *MSc, FICMS*, **Fatima A.S. Al-Rawi**[2] *FICMS*

Abstract

Background: Chronic myeloid leukemia (CML) is a clonal proliferative disorder resulting from neoplastic transformation of primitive hematopoietic stem cell. Many prognostic classifications based on several clinical and biological features had been applied to this disease. The Sokal scoring system is a risk classification system which is frequently used, where according to the hazard ratio three prognostic groups are defined which are referred to as good, intermediate and high risk group. Other parameters including clinical, hematological as well as histological features had been found to have an impact on the prognosis in CML.

Aim of the study: To determine the prognostic significance of several histological features present at the diagnosis including the degree of marrow fibrosis and megakaryocytic proliferation and to confirm the effect of bone marrow histological features on the well-known Sokal's prognostic risk system.

Methods: This study was conducted on sixty-two Iraqi patients with non-blastic CML, where the clinical, hematological and histopathological features were evaluated. Trephine biopsies from all these patients were processed and stained with Haematoxylin and Eosin to determine haemopoietic cell distribution and with Gomoroi's Silver stain for the evaluation of reticulin fibers. The hematological peripheral blood parameters were measured by Multichannel Analyzer Coulter S Plus.

Results: Different variables in trephine biopsy were measured and compared subjectively and quantitatively. The mean haemopoietic tissue was 78.96±17.68 and the mean number of megakaryocytes per mm^2 was 58.2±40.8. When Sokal formula was applied to all cases they were divided into three prognostic groups and were referred to as low (13 cases), intermediate (30 cases) and high risk group (19 cases). There was significant difference among the three groups as the high risk had an aggressive course carrying unfavorable prognostic features.

Conclusion: This study had confirm the reliability of detailed histopathological features of marrow trephine biopsy in patients with chronic phase CML including megakaryocytic number and the degree of fibrosis as they correlate well with clinical manifestation at presentation and thus added newer parameters to the Sokal scoring system in defining risk groups.

Keywords: Chronic myelogenous leukemia, Bone marrow biopsy, Prognosis, Risk groups.

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Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder resulting from neoplastic transformation of primitive hematopoietic stem cell[1-3]. CML has a bi- or tri- phasic course, it usually presents in a chronic phase with an indolent course characterized by an elevated white blood cell count including more primitive myeloid cells normally found only in bone marrow. Since the transformation is unpredictable, the presentation can be in accelerated or in blast phases with complications[4-8].

Prognostic studies in CML at diagnosis have attracted the interest of a number of investigators[9-17]. These attempts at prognostic classification have been based on the use of several clinical and biological features; including age, spleen size, liver size, platelet count, WBC count, blast and basophile percent in the blood or bone marrow, nucleated red

[1] **Dept. Clinical Pathology, College of Medicine, Al-Nahrain University.** [2] **Al-Karama Teaching Hospital.**

Address correspondence to Dr. Fatima Al-Rawi.

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blood cells, and cytogenetic clonal evolution[9-13]. A synthesis staging system has incorporated factors from all these systems and resulting in a simple model for staging which can identify 4 stages with different outcomes[14]. Another frequently used risk classification is Sokal's prognostic risk system[17], where the hazard ratio is derived from the formula = Exp 0.0116 (age-43.4) + 0.0345 (spleen-7.51) + 0.188 [(platelet/700)^2-0.562] + 0.0887 (blasts-2.10). The mean value and range of the variables used in the hazard ratio formula were shown in table 1. Accordingly, three prognostic groups were defined; those with hazard ratio <0.8, between 0.8-1.2 and >1.2 and were referred to as good, intermediate and high-risk group respectively.

The stage at diagnosis has been identified as one of the most important predictors of survival after treatment with interferon Alpha therapy and also predictive for patients treated with chemotherapy alone[18,19]. The response to treatment with interferon Alpha is a significant prognostic factor for long term survival[1,18].

The prognostic capacity of several previously published staging systems did not involve histological features of the bone marrow. On the other hand, a few series, which were predominantly focused on morphological parameters, revealed according to uni- as well as multivariate analysis procedures that, several of these factors exert an influence on survival[20,21]. Many workers have pointed out that myelofibrosis is a poor prognostic feature in CML, chiefly when it appears during the course of disease[22-31].

Aim of the Study

The present study was undertaken with two primary aims: (a) to determine the prognostic significance of several histological features present at diagnosis of disease, including the degree of marrow fibrosis and megakaryocytic proliferation, and (b) to ascertain whether bone marrow histological features may add prognostic weight to the most widely accepted CML prognostic system[17].

Materials & Methods

From January 1985 to December 1993 a retrospective study of 62 patients with chronic myeloid leukemia (non-blast phase) was done, 37 patients were male and 25 patients were female. All cases were collected from Teaching Laboratories of the Medical City. The following variables were estimated in each case:

Clinical features recorded at presentation include: patient's age, sex, fever, sweating, weight loss, anorexia, abdominal fullness, thrombosis, gout, pallor, generalized weakness and easy fatigability, left hypochondrial pain, hemorrhage, presence of lymphadenopathy, liver and spleen size in centimeters below costal margin, and pre-diagnostic symptoms duration.

Haematological parameters were measured by multichannel analyzer Coulter S Plus and blood films evaluated using standard Rowmanowsky dyes.

Bone marrow aspiration, material of 42 cases was evaluated independently of their previous reports for myeloid: erythroid ratio, basophiles, eosinophils, promyelocytes and blasts percentage.

Marrow trephine biopsy, all the biopsies were cores obtained from posterior iliac crests using Jamshidi needles, fixed with Bouin's solution, decalcified, embedded in paraffin, and sectioned. The minimal size of the cores was 2×12 mm. Two sections were obtained each of 5 μm thickness, one stained with haematoxylin and eosin, and the other stained with Gomoroi's silver stain for reticulin fibers.

Biopsy evaluation

Subjective evaluation[32]

(a) Megakaryocyte number:

- Decrease: < 13 MKCs/sq. mm. Whole marrow area.
- Normal: (3-5 MKCs/ low power field) 13-25 MKCs/sq. mm. whole marrow area.
- Slight increase 26-42 MKCs/sq. mm. whole marrow area.

- Moderate increase 43-74 MKCs/sq. mm. whole marrow area.
- Marked increase ≥ 75 MKCs/sq. mm. whole marrow area.

(b) Reticulin

This was evaluated in fashion similar to that employed by others[32-34] and was graded as:

- Normal: no fibers, or occasional individual fine fibers.
- Slight increase: fine fiber network throughout much of the section.
- Moderate increase: diffuse fiber networks.
- Marked increase: diffuse, often coarse, long fibers usually arranged in bundles.

Quantitative evaluation:

(a) Cellularity (haemopoietic tissue)[34,35]

The percentage area of marrow occupied by haemopoietic tissue, fat, and fibrous tissue (H&E and reticulin stain) was measured by "Chalkley point array graticule".

(b) MKCs number and blood vessels: were calculated by using a window with a known surface area inserted in the eyepiece of the microscope[30,36].

(c) Osteoblast index and Average trabecular bone width: were measured by linear graticule (7×) calibrated at each magnification of the microscope[23,37].

Risk groups,

Patients relative risks were calculated according to Sokal formula[17] into:

Low risk : 13 cases
Intermediate risk : 30 cases
High risk : 19 cases

Statistical Methods

- The Chi square test was used for differences in percentage.
- One way ANOVA (analysis of variance) was used for comparing the mean values of the clinical and haematological features.

Results

The mean value of haemopoietic tissue was 78.96±17.68, and the mean number of MKCs/mm^2 was 58.2±40.8. All of the variables included in this study were compared among the three different risk groups.

Table 1: The mean value and range of the variables used in the hazard ratio formula

Variables	Mean±SD	Range
Spleen	8.6±4.3	1-20
Age	44.7±16.4	14-88
Blast (bone marrow aspiration)	3±2.1	0-10
Platelet ×10^9/l	453.0±325.0	101-1405

* Centimeter below the costal origin.

Clinical features (Table 2): cases of high-risk group were older age, and had larger spleen. And among the qualitative parameters; pallor, weight loss, and abdominal pain were significantly higher in the same group, (P<0.05).

Table 2: Clinical features of different RISK groups

	Low risk(13)* Mean±SD	Intermediate risk(30)* Mean±SD	High risk(19)* Mean±SD	P-value
Age (Y)	33.92±11.61	46.10±17.45	49.79±14.80	0.019**
Liver@	2.77±2.49	2.03±2.41	3.53±2.55	0.130[NS]
Spleen@	6.32±2.59	8.14±4.53	10.42±3.44	0.013**
Pre-diagnosis Duration	3.79±4.10	4.89±4.10	8.08±10.30	0.125[NS]

* Number of cases in each stage. ** Significant. @ Cm. below costal margin. [NS]: Not significant.

Haematological parameters (Table 3): high-risk group had significantly lower Hb values, higher WBC counts; higher platelets and higher percentages of basophiles, and immature precursors (blasts and promyelocytes). The frequency of leucoerythroblastic picture was more in high-risk group, as the P-value was <0.05.

Table 3: Peripheral blood of different RISK groups

	Low risk (13)* Mean±SD	Intermediate risk (30)* Mean±SD	High risk(19)* Mean±SD	P-value
Hb g/dl	11.7±1.6	10.1±2.5	8.4±1.4	0.036**
Platelet $\times 10^9$/L	302.2±128.3	411.0±217.9	614.2±464.0	0.017**
WBC $\times 10^9$/L	127.6±173.4	233.4±495.2	267.3±878.9	0.028**
Neutrophil %	54.0±8.1	48.3±14.3	46.9±10.7	0.190[NS]
Eosinophil %	2.1±1.7	2.8±2.3	3.0±2.0	0.488[NS]
Basophil %	3.2±1.9	4.3±3.5	6.26±5.0	0.039**
Promyelocyte %	2.1±1.9	5.3±4.1	5.9±4.6	0.020**
Blast %	0.62±0.65	1.97±1.94	3.95±2.37	<0.001**
NRBC/100WBC	0.52±0.95	0.74±2.35	0.92±1.5	0.739[NS]

* Number of cases in each stage. ** Significant. [NS]: Not significant.

Bone marrow aspirate (Table 4): High-risk group had higher percentage of basophile, blast cells and higher myeloid: erythroid ratio. Although promyelocyte percentage was higher in high risk-group, this had failed to reach a significant level.

Table 4: Bone marrow aspirates of different RISK groups in 42 cases

	Low risk (10)* Mean±SD	Intermediate risk (19)* Mean±SD	High risk (13)* Mean±SD	P-value
Basophile %	2.4±2.1	3.6±2.8	5.8±2.5	0.034**
Eosinophil %	3.6±2.9	3.8±2.9	3.6±2.7	0.979[NS]
Promyelocyte %	4.1±1.7	4.4±2.6	5.1±2.6	0.583[NS]
Blast %	1.2±1.5	3.2±2.4	4.3±2.2	0.045**
M:E ratio* **	19.9±8.44	22.9±10.4	25.7±9.4	0.029**

* Number of cases in each stage. ** Significant. *** Myeloid:Erythroid. [NS]: Not significant.

Bone marrow biopsy parameters (Table 5): high risk group had higher percentage of marrow area occupied by fibrous tissue in both (H. & E.) and reticulin stain. The same group had the greatest values of MKCs/mm^2, blood vessels/100 mm^2, osteoblast index and average trabecular bone width but not significant. Although low-risk group had the greatest value of quantitative cellularity, this was not significant. Among the qualitative features of the trephine sections; only the subjective estimation of fibrous tissue and MKCs were significantly higher in high risk group and the P-values were <0.05 for both.

Table 5: Bone marrow biopsy findings of different RIKS groups

	Low risk (13)* Mean±SD	Intermediate risk (30)* Mean±SD	High risk (19)* Mean±SD	P-value
Q. Cellularity (%)	82.9±14.6	78.4±17.7	74.3±21.2	0.395[NS]
Q. Fatty tissue (%)	4.6±6.8	4.4±6.6	5.6±10.4	0.846[NS]
Q. Fibrous tissue (H.&E.) (%)	10.8±12.47	17.2±16.1	22.1±22.4	0.045**
Q. Fibrous tissue (Reticulin stain)	36.6±29.9	49.7±28.1	53.6±32.6	0.044**
MKC/mm^2	50.2±27.0	60.0±44.1	66.9±45.3	0.123[NS]
Bl. vessels /100mm^2	3061.4±1429.3	3489.7±1349.4	3500.1±1504.9	0.544[NS]
Osteoblast index	0.75±0.29	0.77±0.29	0.79±0.22	0.872[NS]
Average Trabecular Bone Width (μm)	73.6±9.7	80.5±12.4	80.9±14.9	0.120[NS]

* Number of cases in each stage. ** Significant. [NS]: Not significant. Q. Quantitative.

Discussion

Sokal and his colleagues evaluated 813 patients with CML and had founded a median survival of 47 months representing a good risk group of patients[17]. Features that are significant include spleen size and percentage of blasts, age and platelet count above 700×10^9/L, all these four variables were significantly higher in this study. Other variables appear to be significantly higher in high-risk group, like weight loss that may be due to vigorous haematopoiesis abdominal pain as a consequence to larger spleen and pallor related to lower Hb value, while the higher percentage of basophile and immature granulocytes is expected to be a reflection to higher leukocyte counts in this group.

Similar results obtained from marrow aspirates as significantly higher percentage of basophile and immature granulocytes and raised myeloid:erythroid ratio in high-risk group. This was attributed to marked granulocytic hyperplasia and or suppressed erythropoiesis which in keeping with lower Hb value. The larger spleen in high-risk group is likely to be correlated with higher leukocyte counts in this group.

Bone marrow fibrosis in association with higher frequency of leukoerythroblastic picture was prominent in high-risk group which may be in part contributed by extramedullary haematopoiesis. The subjective estimation of megakaryocytes although was higher in high-risk group, it failed to reach a significant level.

The Sokal scoring system[17] can successfully discriminates among the defined risk groups because most of poor prognostic categories are found mostly in high risk group, other parameters, however, that are included in Sokal's formula but proven by other investigators[20] as in this study to be associated with poor outcome and these parameters include: (1) Lower Hb concentration, and reduced erythropoiesis in bone marrow[37], (2) Higher WBC counts[20], (3) Higher percentage of basophile in peripheral blood and bone marrow[12], (4) Fibrosis and increased MKCs in trephine biopsies[38-42]. The anemia and the significant reduction of red cells lineage had a remarkable correlation with survival and this probably reflects the expansion of Ph positive cell clone[20,37]. It has been assumed that the significant fibrosis (moderate-marked increase) is in favor of poor outcome[24], but this finding has to be modified, since after assessment of argyrophilic fiber density by morphometry, even a slight increase in reticulin exceeding the doubling of normal value resulted in a conspicuous worsening of survival[20,39-41,43].

Conclusion

This study confirms the validity of detailed histopathologic features of marrow trephine biopsy in patients with chronic phase CML including megakaryocyte number and the degree of fibrosis as they correlate well with clinical manifestations at presentation and added newer parameters to the Sokal scoring system in defining risk groups.

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THE EFFECT OF PACKED CELL VOLUME ON THE BLEEDING TIME AND PLATELETS IN ANEMIA

Subh S. Al-Mudalal[1] *M.Sc. (path), FICMS (Haem)*, **Saad S. Mansoor**[1] *FRCPath*, **Fitua M. Aziz**[2] *B.Sc. MSc*

Abstract

Introduction: Skin bleeding time was widely used as a measure of platelet vessel interaction in haemostasis, and red cells had an important effect on the platelet interaction with damaged vessels and foreign surfaces. It has been found that there was progressive correction of the bleeding time and platelet adhesiveness in vivo and in vitro when the haematocrit was raised by blood transfusion.

Objectives: This study was done to find the effect of haematocrit on the bleeding time in normal and anaemic subjects who had normal renal function and platelet count above 100×10^9/L. Also the relationship of the platelet count and haematocrit was investigated.

Materials & Methods: Bleeding time, platelet count and packed cell volume were performed on 43 healthy and 29 anaemic individuals who had normal renal function and platelet count above 100×10^9/L. the skin bleeding time was measured by modified Ivy's method using a sterile and disposable spring-loaded device and a sphygmomanometer. PCV and platelet count were measured in duplicate by standard laboratory procedure, whereas blood urea and creatinine were measured by Nissler's method and sodium tungistate respectively.

Results: This study had showed that there was a significant inverse correlation between packed cell volume (PCV) and bleeding time (BT) in anaemic patients who had platelet count above 100×10^9/L and normal renal function. Also there was a negative relation between PCV and platelet counts which was more significant in anaemic patients.

Conclusion: Since it is well known that the increase PCV is associated both with arterial thrombosis and with increased platelet vessel interaction therefore the effect of PCV on both BT and platelet count can be considered as haemostatic mechanism for preventing bleeding and thrombosis.

Keywords: PCV, Bleeding time, Platelet count.

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Introduction

The skin bleeding time is a measurement of vascular and platelets integrity[1]. Platelets adhere to the exposed subendothelial collagen and subsequently to each other to form an aggregate that plug the wound[2].

Many hematological parameters may affect the bleeding time. Such as a low platelet count (< 100×10^9 /L) which may prolong the bleeding time[3], increasing the hematocrit in anaemic subjects may shorten the bleeding time in both thrombocytopenic and non thrombo-cytopenic subjects[4] and increasing the number of leukocytes at a constant hematocrit and platelet count may also shorten the bleeding time[5].

It has been shown that prolonged bleeding time which is a well known complication in uraemic patients is profoundly influenced by the anaemia and that red cell transfusion will improve both the bleeding time and platelet retention on glass beads[6].

The Aim: is to determine the relationship of bleeding time to the packed cell volume (PCV) in normal and anaemic subjects whose platelets count is greater than 100×10^9/L and who have a normal renal function. Additionally, the relation of platelet count to the bleeding time was investigated in those subjects.

[1] Dept. Clinical Pathology, College of Medicine, Al-Nahrain University. [2] Pharmaceutical College, Al-Mustansiriya University.

Address Correspondence to Dr. Subh S. Al-Mudalal

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Patients & Methods

This study was conducted on 72 healthy and anaemic individuals (age range 10-45 years, median= 25 years), male to female ratio was 30/42, who were preoperatively attending a plastic surgery clinic (Table I).

Table 1: The demographic characteristics of the subjects enrolled in the study

	Total	Healthy	Anaemic
Number	72	43	29
Male/female	30/42	15/28	15/14

The criteria for their inclusion:

1. Normal renal function.
2. No systemic haemorrhagic symptoms.
3. Platelet count > 100×10^9/L.
4. No history of taking aspirin or other drugs affecting platelet function within the previous 2 weeks.

A sample of venous blood was collected in EDTA containing tube (1.5 mg/ml) for the measurement of packed cell volume (PCV) and platelet count by standard laboratory procedure[7]. Both variables were measured in duplicate and the mean of the two readings was taken. Blood film and reticulocytes count were done using Leishman stain and Brilliant Cresyl Blue respectively[7].

Serum creatinine and blood urea were measured by sodium tungistate and Nissler's method respectively[8].

According to the packed cell volume (PCV) the subjects enrolled in this study were classify into 4 major groups (Table 2).

Table II: Distribution of cases according to their PCV*

	Group I Total group	Group II Anaemic group	Group III Normal female	Group IV Normal male
Number	72	29	28	15
Male/Female	30/42	15/14	28	15
PCV (%) range	21-50	Male = 31-42 Female= 21-36	36-45	42-50

* Normal values of PCV were based on Ref. No. 9.

The skin bleeding time was measured using a modification of Ivy's method[10]. A sphygmomanometer cuff was placed on the upper arm and inflated to 40 mmHg. The skin of the forearm was cleaned with alcohol and allowed to dry. Duplicate skin punctures were made on the volar surface of the forearm near the anticubital crease using a sterile disposable spring-loaded device (Simplate 11 General Diagnostics). Stop watch was started when bleeding begin and the edge of each puncture was touched with a Whatman No.1 filter paper every 15 seconds until no further blood was absorbed by the paper and the mean of the reading was taken.

Results

A total of 72 healthy and anaemic patients were enrolled in this study (Table 1). They were classified into 4 groups according to their PCV (Table 2). The packed cell volume (PCV), bleeding time (BT) and platelets count (PT) were measured and the mean ±SE, median and the range of these variables were calculated (Table 3). It has been found that although the mean PCV was lower in females and the mean BT and platelet count was higher in females, but the comparison of these variables between sexes was insignificant.

Table 3: The PCV, BT and PT of all cases

		Number	Mean ± SE	Median	Range
PCV (%)	Total Male Female	72 30 42	38±0.544 40.27±0.92 37.8±0.62	38 40.13 38	21-50 31-50 21-45
BT (min.)	Total Male Female	72 30 42	2.9±0.18 2.7±0.18 2.8±0.17	2.5 2 2.5	1-7 1-5 1-7
Plat. ($\times 10^9$/L)	Total Male Female	72 30 42	206±4.34 193±7.42 215±4.8	200 200 200	120-339 120-329 132-339

On examining the blood film, all anaemic subjects had hypochromic microcytic anaemia and the range of the corrected reticulocyte count was (0.5-1.2 %). The blood films morphology were suggestive of iron deficiency anaemia. The WBC and differential count was normal. Platelet counts were above 100×10^9/L with normal morphology (Table 3). All the individuals in the 4 groups had normal blood urea and serum creatinine levels.

The relationship of the bleeding time to PCV was demonstrated in Table 4, Figures 1 and 2, showing a significant ($P<0.05$) and highly significant ($P<0.01$) correlation in group I (total group) and group II (anaemic group) respectively. However, when sexes were analyzed separately there was an insignificant inverse correlation in group III and IV which include normal males and females.

Table IV: The correlation of the packed cell volume with the bleeding time and with the platelets count (r=regression correlation)

The Groups	Number	PCV % and BT (min.)	PCV% and platelets x10^9/L.
Group I (Total group)	(n=72)	-0.238*	-0.389*
Group II (Anemic Group)	(n=29)	0.606**	-0.541**
Group III (Female Group)	(n=28)	-0.004	-0.13646
Group IV (Male Group)	(n=15)	-0.051	-0.1082

* $P < 0.05$, ** $P < 0.01$

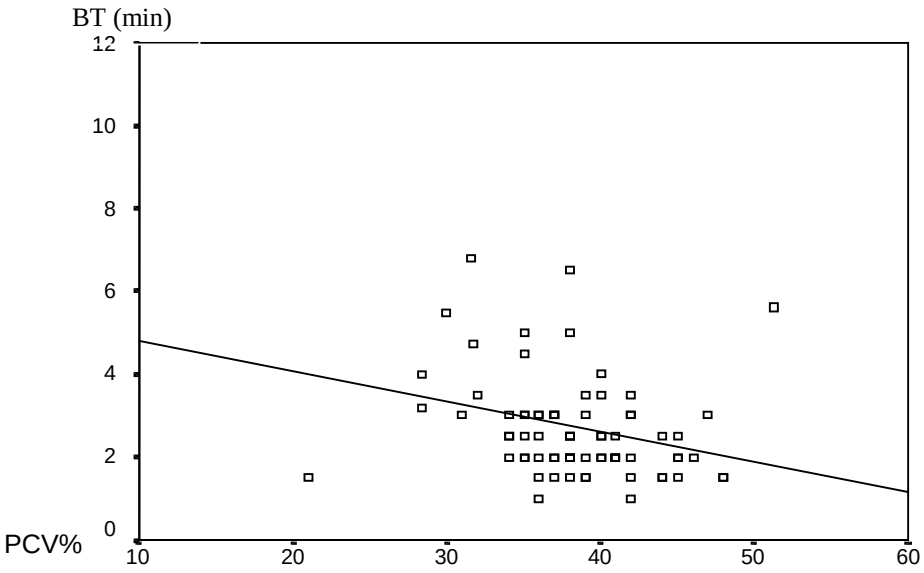

Figure I: Correlation of BT to PCV in group I (total group, BT= bleeding time; PCV= packed cell volume

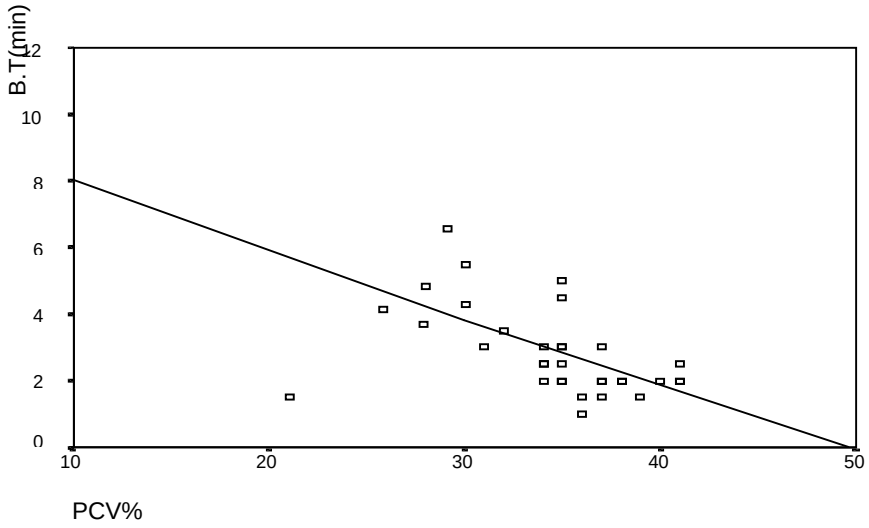

Figure II: Correlation of BT to PCV in group II (anaemic group). BT= bleeding time; PCV= packed cell volume

Similarly, by correlating the platelet count to the PCV there was a significant inverse correlation in group I and II while in group III and IV it was insignificant, (Table IV, Figure III and Figure IV).

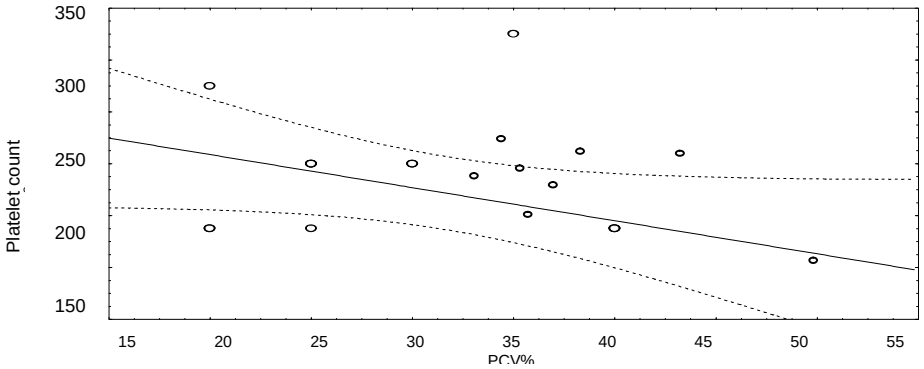

Figure III: The correlation of PCV to platelet count in total group

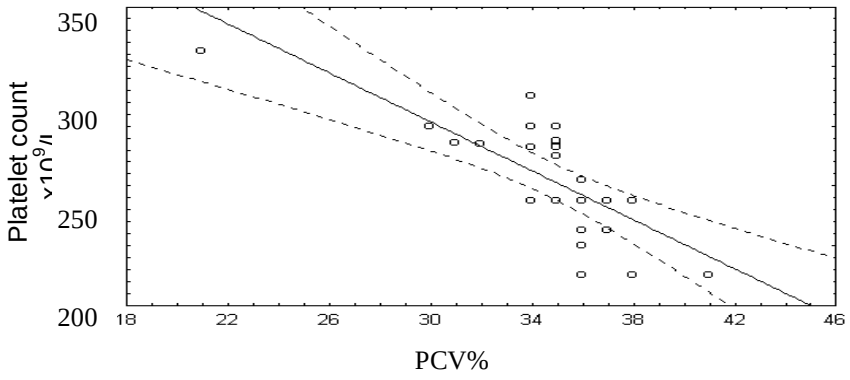

Fig IV: The correlation of PCV to platelet count in anaemic group.

Discussion

Low hematocrite is an often neglected cause in the pathogenesis of prolonged bleeding time in an anaemic patient. Many studies revealed a linear negative correlation between PCV and bleeding time in patients with platelet count below 100×10^9 /L[11-13], however, little information is available on those with platelet counts above 100×10^9 /L.

The present study had confirmed the effect of PCV on the bleeding time in patients with platelet count above 100×10^9/L and normal renal function. These results were proposed to be attributed to the effect of red cells on the adhesion of platelets to the damaged blood vessels and their subsequent aggregation to form a hemostatic plug[15].

Bonea et al[11] had proposed that since the flow of blood in small blood vessel may cause physical dispersion of platelets toward the subendothelial surface thus promoting its interaction with the wall of the blood vessel. Also following injury to the small blood vessel, RBC will activate platelets by releasing small amount of adenosine diphosphate (ADP) into the microvasculature following the hemolysis that often occur during hemostasis[11,15,16]. Furthermore, it is well-known that the red cells bind to prostacycline, hence the increase in the red cell mass will enhance platelets adhesion and aggregation by removing prostacyclin which inhibit platelet adhesion and aggregation[14,17].

Blajchman et al[4] had confirm that anaemia would contribute to the prolong bleeding time independently on platelets count, and that RBC transfusion was capable of shortening BT in thrombocytopenic anaemic animal[4].

However, in normal male and female groups there was an insignificant correlation between the PCV and BT, which was expected since they had a narrow range of PCV values. This confirms the finding of Small M. et al. and Lino et al[14,18].

Bleeding is a well-known complication of uraemia and many studies had shown that increasing hematocrit with either red cells (RBCs) transfusion or erythropoietin will correct the prolonged bleeding time which is often seen in those patients[19,20]. This was explained by the effect of increasing the red cell mass[19], in addition to correcting platelets function[20]. In agreement with Small et al[14] and other studies which had shown that there was a significant negative correlation between PCV and platelet count which may be explained by the effect of low PCV on the secretion of erythropoietin causing stimulation of megakaryocytes and hence, increases platelet count[21].

In normal female this correlation was insignificant since most of those females were at the child-bearing age who usually had fluctuation in the platelet number during their menstrual cycle[7,22]. While in male the narrow range of PCV value may explain the insignificant correlation between platelet count and PCV.

It had been observed that patients who are liable for thrombosis, such as those with atherosclerosis and hyper-cholesterolaemia, had an elevated mean hematocrit[23] and a shortened mean bleeding time[24]. Therefore it is possible that the high PCV not only increase blood viscosity but may also promote platelets adhesion and aggregation to initiate thrombosis.

Conclusion

Since it is well known that the increase PCV is associated both with arterial thrombosis and with increased platelet vessel interaction therefore the effect of PCV on both BT and platelet count can be considered as haemostatic mechanism for preventing bleeding and thrombosis.

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CANCER AND DIETARY HABITS

Amal S. Khudair[1] *FICMS*, **Ahmad A. Al-Azawi**[2] *FICMS*,
Abdul-Hussain M. Al-Hadi[1] *Ms.C. Ph.D.*

Abstract:
Background: Cancer is second only to heart disease as a cause of death in Westernized countries. Up to 80% of all cancer have a link to diet.

Objectives: To explore dietary habits of relevance to cancer risk, some demographic characteristics, and nutritional status assessment of a sample of cancer patients.

Methods: A group of cancer patients have been interviewed and examined for demographic characteristics, anthropometrics, and dietary and supplementary intakes.

Results: The studied sample showed rare intake of fish by (35.5%) of patients and once per month by another 35.5%. Olive oil intake was rare by (71%) of patients. Fruit and vegetable were taken daily by (37.1%) and (51.6%) respectively. Grade one and grade two obesity have been shown by (35.5%) and (25.8%) respectively.

Conclusion: Many dietary habits have been unfavorable, and a health education program is recommended to promote healthful dietary habits. Increasing each of fish, olive oil, fruits and vegetables, with decreasing red meat and saturated fat intake should be encouraged for controlling cancer promotion. Weight management need to be emphasized too.

Key words: Cancer, Diet, Dietary habits, Nutrition, Nutritional status assessment.

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Introduction

Cancer is second only to heart disease as a cause of death in westernized countries. A number of observations strongly suggest that environment can influence cancer risk[1]. Environment is broadly defined to include a wide range of life style factors, including diet, social and cultural practices, with some of these being poorly specified[2].

One estimate of the proportion of all cancers attributable to diet ranges from 10-70 %, with an overall average of 35%. Up to 80% of all cancers may have some link to nutrition[2]. This compares with 25-40 % from tobacco and 2-8 % from occupational exposure[1].

Cancer development is thought to be a several stage process of initiation, followed by promotion. The initiation step is brought about by carcinogen which are widespread in the environment and which cause change in the DNA of cells. Before malignancy develops, another stage, promotion is necessary[1-3]. In general, diet is thought to be particularly important in the promotion of cancer and in protection against the effects of carcinogens rather than as a carrier of carcinogens themselves[1].

Nutrition plays an important role in the care of the person with cancer, from diagnosis onwards. Provision of adequate nutrition makes a major contribution toward the clinical, biochemical and psychological status of the cancer patients in the face of the disease process[1,2,4]. Nutritional support may also help to reduce the incidence and severity of undesirable adverse effects of treatment and so improve outcome.

Objectives

1. Exploring dietary habits related to cancer risk, in order to modify unfavorable one through nutrition education program.
2. Evaluating the nutritional status, diet history, other patients characteristics, and

[1] **Dept. Community Medicine,** [2] **Section of Oncology, Dept. Medicine, College of Medicine, Al-Nahrain University.**
Address correspondence to Dr. Amal S. Khudair.
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supplementation therapy so that to improve management and outcome.

Subjects & methods

In a descriptive type of study (cross-sectional),a sample of 62 patients with different types of malignancies, attending the oncology clinic at Al-Kadhimyia Teaching Hospital have been included in this study. All adults attended the oncology clinic on Monday have been included from 4th March until 24th June 2002 for feasibility purposes.

Special form, desingned by the authors, has been used including age, sex, educational status, marital status, history of diabetes mellitus, hypertension, and myocardial infarction, family history of malignancy and the type of the malignancy, presence of metastasis, mode of treatment, history of smoking and alcohol, dietary history by food frequency inquiry, and anthropometric measurements.

Body mass index and waist hip ratio has been used for nutritional status and central obesity assessment respectively[5]. Hemoglobin level in Gm/dl has been used as a basic biochemical nutrition indicator. The cut-off point for diagnosis of anemia has been (13.5) and (12) Gm/dl for males and females respectively[6].

EPI-6 computer program has been used for entry and analysis of data. Regression, Z-test and chi-sequare has been used whenever applicable, p value less than 0.05 considered statistically significant.

Results

A group of 62 patients, 26(42%) males and 36(58%) females were studied. Their age ranged from 20-74 year with a mean (+S.D) of (49.7+12). Mean age (+S.D)of males and females was (52.5+13) and (48+11) year respectively.

In regard to marital status 16 (26%) were single, 44 (71%) were married and 2 (3%) were widows. Marital status in relation to sex is shown in table 1.

History of diabetes mellitus was positive in 9 (14.5%) patients, hypertension in 12 (19%), and myocardial infarction in 4 (6.5%) patients.

Family history of diabetes mellitus, hypertension, and myocardial infarction is shown in table 2. Family history of malignant diseases was positive in 4 (6.5%) patients. Affected relatives have been mother, father, brother and sister.

Smoking has been positive in 6 (10%) patients, while ex smoking was positive in 12 (20%) patients. Vitamin and mineral supplements have been taken by 8 (13%), and 5 (8%) patients respectively. Nutritional status assessment, using body mass index values is shown in table 3.

Central obesity was positive in 56% of males (WHR>0.8) and in 81% of females (WHR>1)[5]. Nutritional status of patients with malignant diseases was not dependent on their educational status as determined by correlation analysis (r=-0.01). Anemia was detected in 24 (92.3%) males and 30 (83.3%) females (p=0.2).

Dietary history, using frequency of intake of some food items, results is shown in table 4. Types of cancer: Twenty two (34.5%) patients had breast cancer, 9 (14.5%) patients had bladder cancer, 8 (12.9%) patients had colon and colorectal cancer, 5 (8%) patients had lung cancer, 2 (3.2%) patients had renal cancer, 2 (3.2%) patients had stomach cancer, and 1 patient for each of intestinal, uterine, hepatic, choriocarcinoma, laryngeal cancer, ovarian, seminoma, leukemia, lymphoma, prostate, rhabdomyosarcoma, cervical lymph nodes, and liver secondary of unknown origin.

Breast cancer cases constituted the largest proportion among total sample (35.5%), and among females (61%). Mean age (+S.D) of female with breast cancer was (45.36+10.03) year. Mean (+S.D) education years was (8.25+5.36) year. Six (27.3%) patients were single, and 16 (72.7%) were married. Obesity was shown in 41%, overweight in 18% of them. Family history of cancer was

positive in one case, father with liver cancer.

Metastases was positive in 12 (54.5%) cases, the mean age (+S.D) was (44.3+8.54) year. All breast cancer patients showed a negative history for smoking and alcohol consumption. Dietary history of breast cancer patients is shown in table 5. Quantitatively, bladder cancer came second among the sample. Males predominated, as the male to female ratio was 8:1. Mean age (+S.D) of patients was (53.4+9.54). None of cases had a family history of cancer of any type. Smoking was positive in 3 (33.3%) cases; in addition to 3 (33.3%) cases as ex-smokers.

Only one patient gave positive history of alcohol consumption. Dietary history in terms of frequency of intake is shown in table 6.

Six (9.67%) colon cancer cases in addition to 2 (3.22%) colorectal cancer constituted the third rank among types of cancer. Mean age (+S.D) of patients was (47.87+11.76) year, male to female ratio was 1. Mean (+S.D) education years was (7.75+6.45) year. Family history was positive in one female patient; her sister was affected by colon cancer too. Current smoking was positive in one male patient (12.5%), in addition to 2 (25%) males ex-smokers was noticed. None of the patients consume alcohol. Dietary history of patients with colon and colorectal cancer is shown in table 7.

Table 1: Distribution of patients by marital status and gender

Marital status	Males		Females	
	Number	%	Number	%
Single	8	31	8	22
Married	18	69	26	72
Widow	0	0	2	6
Total	26	100	36	100

Table 2: Distribution of patients by positive family history of non-communicable diseases

Disease	Mother		Father	
	Number	%	Number	%
Diabetes mellitus	7	11.3	5	8.1
Hypertension	16	26	10	16
Myocardial Infarction	2	3.2	5	8.1

NOTE: n=45

Table 3: Nutritional status assessment of patients with malignant diseases according to sex

Body mass index	Males		Females		Total	
	Number	%	Number	%	Number	%
Less than 18.5 (Undernutrition)	1	3.8	1	2.8	2	3.2
18.5-24.9 (normal)	7	27	15	41.6	22	35.5
25-29.9 (grade I obesity)	16	61.5	6	16.6	22	35.5
30-39.9 (grade II obesity)	2	39	14	39	16	25.8
40 and above (grade III obesity)	0	0	0	0	0	0
Total	26	100	36	100	62	100

P=0.001

Table 4: Distribution of patients by dietary intake

Frequency	meat	Chicken	Fish	fruits	Vegetables	Olive oil
	Percentages of patients					
Daily	11.2	0	0	37.1	51.6	9.7
3/week	13.2	16.1	3	11.3	4.9	1.6
2/week	22.5	35.5	10	14.5	13	1.6
1/week	22.5	29	16	32.3	30.6	9.7
Monthly	24.2	17.8	3.5	3.2	0	6.4
Rarely	6.4	1.6		1.6	0	71
Total	100	100	100	100	100	100

Table 5: Dietary intake history of breast cancer cases

Frequency	meat	Fish	Olive oil	fruits s	Vegetable
	Percentage of patients				
Daily	13.63	0	18.2	50	59
3/week	9.1	4.5	0	4.5	0
2/week	36	4.5	4.5	13.5	9
1/week	18	18	4.5	32	32
Monthly	23	41.36	4.5	0	0
Rarely	0	27.27	68.2	0	0

NOTE: Total=22 cases

Table 6: dietary history of bladder cancer patients

Frequency	Meat	Chicken	Fish	fruits	vegetables	Olive oil
	Percent of patients					
Daily	22.2	0	0	55.5	55.5	0
3/week	22.2	11.1	0	0	0	11.1
2/week	0	33.3	11.1	11.1	22.2	0
1/week	22.2	44.4	11.1	22.2	22.2	22.2
Monthly	22.2	0	33.3	11.1	0	0
Rarely	11.1	11.1	44.4	0	0	66.6
Total	100	100	100	100	100	100

Table 7: Dietary history of patients with colon and colorectal cancer

Frequency	meat	Chicken	fish	fruit	vegetables	Olive oil
Daily	0	0	0	12.5	12.5	12.5
3/week	0	12.5	0	0	0	0
2/week	12.5	12.5	0	12.5	37.5	0
1/week	37.5	50	0	62.5	50	0
Mothly	50	25	0	12.5	0	0
Rarely	0	0	62.5	0	0	87.5
Total	100	100	37.5	100	100	100

Discussion

Food frequency questionnaire method has been used for feasibility purposes. Record methods although more accurate, but not applicable because of psychological state of cancer cases. Per capita fat consumption could not be calculated because, fat consumption was unknown by most of patients probably for the same cause.

In the studied sample, obesity and overweight seemed to be a problem despite the fact that many cancer cases may lose weight because of the disease itself. Obesity is a risk factor for many types of cancer including breast cancer, colon cancer, and prostate cancer[1,2,4].

Red meat is suspected of increasing the risk of cancer particularly colorectal[1,4,7] but red meat consumption among the studied sample favored a low frequency in general; colon and colorectal cases in particular showed even a lesser intake of red meat. This may be due to economic causes rather than to awareness of the risk.

Unfortunately, fish intake seemed to be scarce. Among many health benefits of regular fish intake is the suppression of tumor promotion related to w3 polyunsaturates from alpha-linolenic acid found in fish oil[1,2,4].

Fruits and vegetables intake did not show high frequency among the majority of cases. One of the most consistent observations in epidemiology is that increased fruit and vegetable intake is protective against the development of a wide spectrum of precancers and cancers[1,8-10]. In addition to vitamins C, E,

beta-carotene, and non-starch poly-saccharides (NSP), numerous coloring and flavoring substances have been identified in fruits and vegetables. Many of them are pharmacologically active chemopreventive agents[9,11]. The concept of using micronutrient for chemoprevention of cancer is based on the evidence from human epidemiology, the results of a few clinical trials, and studies of animal carcinogenesis models for cancer inhibiting potential of these substances[10].

Higher fiber intake is associated with low plasma levels of all major biologically active sex hormones[12-14]. This decreased hormone bioavailability may reduce the risk of hormone dependent cancers such as breast cancer. Fruit and vegetable intake among breast cancer cases in the studied sample is less than daily in about half of them. In two recent case-control studies in Spain and Greece, it has been found that women who used more olive oil had reduced risk of breast cancer[15,16].

Unfortunately in the studied sample breast cancer cases showed rare intake of olive oil. The extent to which cancer risk is attributable to genetic factors varies among tumor types. For rare childhood cancers up to 30% occur in genetically predisposed individuals, where as not more than 5% to 10% of common adult cancers arise in an hereditary setting[7,16]. The studied sample showed a similar result as 6.45% of cases had a positive family history. Of these one case (1.6%) only with the same type of cancer, i.e., colon cancer. In a study conducted by Nagi B. Kumar et al, a family history of cancer was recorded in 65% of the patients[1,7]. This big difference in family history of cancer from the studied sample may reflect differences among populations in environmental factors related to cancer risk. It may also reflect a change in environmental factors related to cancer in Iraq that caused the current cancer prevalence rate to exceed the rate of predecesent people.

Vitamin and mineral intake by the studied sample is very small (13% and 8% respectively) in comparison to multivitamin and mineral intake reported by Nagi to be (58.6%)[17]. High prevalence of anemia observed among the studied sample, vitamin C deficiency that has been described by many investigators[18] among cancer patients may rationalize the use of mineral/vitamin supplements.

Conclusion & Recommendations

In the studied sample many nutritional and dietary problems relevant to cancer risk have been elicited. High prevalence of overweight and obesity, a very well known risk factor for many types of cancer, necessitate increase public awareness of the importance of weight management through scientific approach. Healthy diet promotion may be a priority in cancer prevention. Most cancers have a latency period of 10-20 years, which provides ample time for prevention measures[11]. A health education dietary program may be started to overcome bad environmental influence. Among the healthful dietary recommendations is to decrease saturated fat and red meat, and to increase fish, olive oil, and fruit and vegetable. Medical personnel may prescribe multivitamin and mineral to cancer patients accordingly.

Patient's management may get improved in terms of anthropometric nutritional status assessment, appropriate biochemical investigation, nutrition support therapy, and psychological support therapy.

Further studies like dietary evaluation of cancer patients using record methods in correlation approach; chemoprevention intervention trials may be tried on certain eligible cases.

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MEDICOLEGAL STUDY OF MASS HYOSCINE POISONING

Tarif S. Al-Gurairy[1] *C.E.S., M.Phil*, **Faik A. Bakir**[2] *D.T.M. & H., D.M.J.Path.*, **Mutaz A. Al-Qazzaz**[1] *M.Sc., FICMS*

Abstract

Background: Food poisoning includes a number of disorders mostly bacterial in origin causing acute gastroentrites developing within 48hours of ingestion of food or drink. It may also be due to intestinal allergy to shellfish or eating unripe fruit, unsuitable foods or poisonous mushroom and sometimes a chemical poison in food may be consumed. However poisoning with hyoscine is a rare incident.

Objective: The study was conducted in order to identify the type of poisonous material consumed and evaluate its pathological and toxicological effects.

Methods: The study was carried out on 15 people with sever poisoning symptoms after having a meal called Dolma. Three were died after different intervals and were submitted to postmortem examination with histopathological and toxicological analysis using thin layer chromatography (TLC) and gas chromatography (GC) apparatus.

Results: In out of 15 people with history of eating cooked food which was prepared by mistake form poisonous plant, 3 children died under the age of eleven whereas the remainder was having gained recovery after 6 to 24 hours. Autopsy on the 3 dead children revealed petechial hemorrhages in the stomach, brain, heart and lungs. Histologically there were congestion and local hemorrhagic spots in the brain and lungs with fatty changes and massive necrosis in the liver.

Conclusion: The study showed that the effect of such a poison directly depends on both the amount of poison ingested and the victims' age indicating the vulnerability of infants and young children. It was also concluded that the poison producing an anoxic state accompanied with chemical damage in the capillary walls causing these hemorrhagic spots with cell necrosis as a result of interference with oxidative production of energy even at low concentration.

Key words: Hyoscine poisoning, Scopolamine toxicity, Atropa belladonna, Datura Stramonuim.

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Introduction

Hyoscine (scopolamine) is an alkaloid derived from certain plants especially dried leaves or leaves and flowering tops of Atropa Belladonna and Datura Stramonium(Jimson Weed)[1].

It is structurally related to atropine but differs chiefly in being a CNS depressant[2]. It is a potent anticholinergic agent blocking competitively the binding of acetylcholine to the receptors at the postganglionic cholinergic endings and has been used for centuries as a drug for the prophylaxis of sea sickness and in prevention of nausea and vomiting in cases with inoperable bowel obstruction[3-5].

Hyoscyamus Nigra and Datura Stramonium contain 0.25-0.5% of atropine or related alkaloids.The lethal dose of hyoscine for adults is about of 30 mg while for children it may be as low as 10 mg[6]. Ingestion of enough hyoscine from the plant dilates the pupil over a period of days[7], where as higher doses may cause sever mydriasis, hot dry skin, dry mouth, rapid pulse and respiration, urinary retention, muscular stiffness, restlessness, anxiety, mania followed by depression and coma[2,8].

Methods

A medicolegal and toxicological study was carried out on 15 people with severe poisoning symptoms after having a meal called Dolma.

[1] Section of Forensic Medicine, Dept. Clinical Pathology, College of Medicine, Al-Nahrain University, [2] Medicolegal Institute, Minstry of Health, Baghdad.

Address correspondence to Dr. Mutaz A. Al-Qazzaz.

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Out of 15, three children died after different intervals. They were submitted to to postmortem histological and toxicological analysis. The methods used in toxicological analysis were TLC and GLC with acidified iodoplatinate reagent detector[9,10] as follows :

About 200 g of minced food and stomach contents were acidified with tartaric acid in separated conical flasks and homogenized in a blender with 400 ml of methanol.

An oliquot of alkaline chloroform extract was evaporated and the residue used for TLC and GLC. The extraction procedure was completed according to the Modified Stass Otto Method[10].

Samples were taken for toxic analysis from stomach contents, blood, liver and kidneys (preserved in alcohol) as well as cooked food.

Systemic screening investigation for searching was done. Samples were taken for histological examination from brain, lung, liver and sections stained by H &E stain.

Results

In fifteen people a history of eating cooked food which was prepared by mistake from poisonous plant similar to the of spinachbeet which is the main vegetable in the preparation of the well known food called Dolma.

Such poisonous plant naturally growing in the fields of northeast and middle of Iraq.

All the victims were suffered from varied poisoning symptoms as it is shown in table 1.

Table 1: The demographic pictures of the victims

Patient no.	Sex	Age	Clinical presentation	Time interval	Outcome
1	Female	10 months	Coma	6 hours	Died
2	Male	1 year	Coma	10 hours	Died
3	Male	10 years	Coma	5 days	Died
4	Male	4 years	Unconscious	25 hours	Alive
5	Male	5 years	Unconscious	25 hours	Alive
6	Female	7 years	Unconscious	24 hours	Alive
7	Female	8 years	Unconscious	24 hours	Alive
8	Male	8 years	Unconscious	24 hours	Alive
9	Male	12 years	Unconscious	20 years	Alive
10	Female	25 years	Unconscious	12 hours	Alive
11	Female	31years	Conscious	6 hours	Alive
12	Male	33 years	Conscious	6 hours	Alive
13	Male	35 years	Conscious	6 hours	Alive
14	Male	39 years	Conscious	6 hours	Alive
15	Female	42 years	Conscious	6 hours	Alive

The clinical presentation of poisoned people varied but 3 children aged 10 months, 1 year and 10 years were seriously ill and presented with coma and 7 cases with unconsciousness whereas the remainder presented with moderate clinical symptoms such as dry mouth, blurred vision, rapid pulse and respiration, abdominal pain but with complete recovery after 6 hours interval. All sever cases of children showed similar clinical features included fever, skin rash, hot dry skin, irritability, convulsions, mydriasis and visual disturbances. They had complete recovery after about 24 hours of admission to hospital.

Postmortem examination of the 3 dead children showed food material in their GIT with petechial hemorrhages seen in the stomach, brain, heart and lugs as well as congestion in all the organs.

Histological examination of the samples taken from certain organs stained with H & E stain showed congestion and local hemorrhagic spots in the brain, lungs as well as fatty changes with massive necrosis of the liver.

Toxicological examinations on stomach contents, blood samples with liver and

kidneys (preserved in alcohol) were submitted using systemic screening investigations for searching (drugs, pesticides, heavy metals).

Two violet spots were obtained on silica gel TLC at RF of 0.19 and 0.01 from the stomach contents and food extract only which were confirmed by positive atropine control.

The solvent system used was: MEOH / NH4OH (100/ 1.5). Detection was done using acidified iodoplatinate reagent[9,10].

Authentic atropine sulfate was injected on the same GC column and similar results were detected, however nothing was detected from liver, blood and kidney samples extracts. The following conditions for GC were used:

1.5 m x 4 mm of 3% OV – 17 on 100 – 120 mexh gas chromatography Q operated at 250 C.

Nitrogen carrier gas with flow rate 40 ml / minute and the relative retention times to cocaine were calculated. The results were o.19 and 1.0 as seen on the GC trace[11].

Discussion

Human injury by plant materials externally coated or internally observed, injected, inserted or inhaled may be accidental or deliberate, self induced or mistakenly, unwisely or maliciously administered by another part[12].

Sever reactions and even fatalities often resulted from over doses of dangerous plant preparations and from toxic plants mistakenly gathered of inadequately prepared for eating[10]. Some poisonous plants in Iraq are frequently encountered like mushroom and well documented fatalities from their eating accidentally by humans and animals but poisoning by other plants such as Atropa Belladonna and related alkaloids are almost rare and because of some difficulties encountered for investigation of such poisoning. People in this study were by mistake used some poisonous plant in order to prepare one of the well known food called Dolma which is prepared from leaves of spinach beet, grapes or other vegetables filled by meat and rice such poisonous plant naturally growing in the fields of northeast and middle of Iraq which is consisting of shrub of 50-120 cm height with milky sap and oval green leaves similar to that of spinach beet. Such plant called Kazaklan or Alocassia[12]. The study showed different clinical features including moderate to sever symptoms for 12 patients who had complete recovery after various intervals as it is shown in the previous table. Out of 15 patients 3 children aged 10 months, 1 year and 10 years were died after 6 hours, 10 hours and 5 days respectively. The effect of such poison depends on both the amount of poison ingested and the victim s age. These two factors might have played a role in their conditions[4].

However the study indicated that infants and young children are more vulnerable than those of other ages. This is a reflection of general vulnerability of these subjects by hyoscine and related alkaloids toxicity even in low concentrations[13].

Toxicological investigations also showed the presence of hyoscine in stomach contents and cooked food extract only and no trace of such poison had been detected in the liver and blood samples .This is supported by the idea that such drugs are extensively metabolized and their blood concentrations fall rapidly after ingestion[1].

Postmortem histological studies showed the presence of pulmonary hemorrhages in the three dead children .The investigation showed a numerous number of petechial hemorrhages which were observed in tissues other than the lungs, including the surface of the heart, brain and gastric mucosa. These hemorrhages might be occurred as a result of anoxia and chemical damage to the capillary walls reaching a sufficient level and giving rise to hemorrhages[14].

Histological study has also showed necrosis and fatty changes in the liver. These might be produced by the effect of chemical substances even at low concentration which are quickly causing

cell death by interfering with oxidative production of energy and rapid hepatocellular injury causing necrosis[15] as well as playing a central role in disturbing the metabolism of many drugs and chemicals.

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AN ULTRASONOGRAPHICALLY SOLID HYDATID DISEASE OF THE LIVER: NEW SONOGRAPHIC SIGN.

Nazar T. Makki[1] *FRCS*, **Adel H. Al-Qaysi**[2] *DMRD Ph.D.*, **Yarub I. Khattab**[3] *FICMS*

Abstract

Objective: To evaluate the significance of tramline sign as a diagnostic and differentiating sign of solid type of hepatic hydatid disease from other solid hepatic tumors.

Methods: The sonographic findings of 331 solid liver masses in 179 patients were evaluated. The tramline sign, which was suggested to be diagnostic of solid hydatid disease, was prospectively analyzed and compared with the result of postoperative or aspiration pathological studies.

Results: The tramline sign was noticed in 18 cases of pathologically proven solid hydatid cysts out of the 331 solid liver masses and not in any of the other masses.

Conclusion: This sonographic sign should prompt us to differentiate solid hydatid cyst from other primary or secondary solid masses.

Key words: Coiled tramline sign, Echinococcal cyst, Liver sonography, Solid hydatid cysts

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Introduction

Hydatid disease is a parasitic infestation caused by tapeworm larvae. Two forms of tapeworms produce most hydatid disease in man, *Echinococcus granulosus* and *Echinococcus multilocularis*[1], the former being the commonest one and produces symptoms by expansion and compression of adjacent structures. It is endemic in the Middle East countries including Iraq. Human is an accidental intermediate host of the parasite and the liver acts as the initial filter andis the most common organ involved in man (60%)[2].

The diagnosis of hydatid disease in the abdomen is usually made by means of sonography and it really represents an important problem in countries where this disease is endemic. It is also interesting problem because of its varied sonographic manifestations.

The sonographic aspect of these cystic formations has been amply studied, giving rise to many typical and frequently diagnostic patterns, thus making further examinations (e.g. CT and MRI) unnecessary[3-12]. However, a relatively small number of cysts appear to be predominantly or completely solid[13-15] and it become difficult, although very important, to distinguish them from primary or secondary solid hepatic tumors. This report describes a new sonographic sign, which was suggested to be a diagnostic and differentiating sign of solid hydatid disease from other solid liver tumors and evaluate its significance in the preoperative diagnosis correlated with the post-operative pathological diagnosis.

Materials & Methods

Between March 1998 and November 2002 a prospective study was made of (179) patient with 331 solid liver masses (solitary in 137 patients and multiple in 42 patients). The patients were referred for sonographic re-evaluation or for US guided needle aspiration biopsy. All were reported as non-echinococcal solid liver tumors (e.g. hemangioma, focal nodular hyperplasia, primary Hepato-Cellular Carcinoma (HCC) or liver secondary). No history of previous medical therapy for hydatid disease or serological test for

[1] Dept. Surgery, [2] Dept. Surgery-Division of Radiology, [3] Dept. Clinical Pathology, College of Medicine, Al-Nahrain University.

Address correspondence to Dr. Adil H. Al-Qaysi. E-mail: adelqaysi@yahoo.com

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diagnosis of hydatid disease. In most cases sonography was the only method of diagnosis. Sixteen patients had computed tomography with contrast enhancement and magnetic resonance imaging was performed in 7 cases only. The tramline sign was used as the only criterion for the differentiation. There was surgical and/or US guided aspiration pathological confirmation in all cases. All examinations were performed using high resolution scanner (Siemens, Sonoline Versa Pro, Siemens Company, Erlangen, Germany) using 3.5 MHz curved transducer. All patients fasted overnight as the only preparation.

The surgical and pathological reports were compared with the sonographic findings. Medical reports were also reviewed to determine the patient's clinical presentation.

Results

The tramline sign, which was suggested to be characteristic of solid hydatid disease, was noticed only in (18) masses out of the 331 masses (16 solitary and tow multiple masses). Mean age of the patients was 33.4 years (range was 19-11 were males and 7 female. The majority of the patients complained from right upper abdominal discomfort. Only 3 patients were asymptomatic.

Sonography showed well-defined round or oval-shaped masses (6-12.3 cm) (mean diameter was 9.1 cm) with smooth and regular outlines. All were with hyper or isoechoic (homogeneous or inhomogeneous) internal architecture compared with the adjacent liver parenchyma with two parallel coiled hypoechoic linear structures seen within the mass (coiled tramline sign) (Figure 1). There was internal snowstorm pattern of high level echoes on the isoechoic background on 5 cases only (27.8%). Depending on this appearance a diagnosis of solid pattern hydatid disease was made.

The surgical reports proved the sonographic diagnosis and showed, in the majority of cases, that the evacuated cyst contained very thick mucus or yellow gelatinous material more or less similar to the purulent material mixed with the membranes. Pathological examination of the open cysts showed fibrous laminar layer and an inner germinal layer which was irregular, fragmented and also contained foci of necrosis and mixed chronic inflammatory cell infiltration. Cytologically we diagnosed echinococcosis by identification of scolices and / or tooth- shaped hooklets on a clear background of the smear.

Correlation between final pathological and / or cytological reports diagnosed and the prior ultrasonic pathognomonic and diagnostic sign's of solid hydatid disease of the liver. Revealed a sensitivity and specificity and over all diagnostic accuracy of 100%, no false positive case reports are recorded, P value (< 0.05).

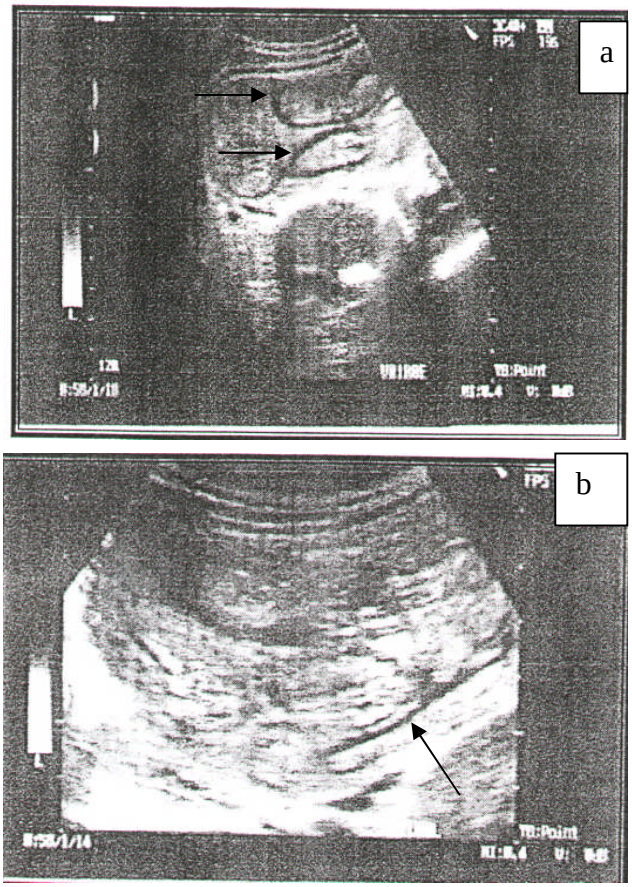

Figure 1 (a & b): Transverse subcostal view of the liver showing two parallel hypoechoic lines (tramline sign) as denoted by the arrows

Discussion

The Ultrasonographically solid appearance of hydatid disease is not a very rare finding, Francisco et al[16]. The real diagnostic difficulties in the diagnosis of solid hydatid disease arise from primary hepatic neoplasms and metastases. Ultrasound patterns of such lesions can be quite similar to those described in primary and secondary liver lesions and a trial to describe a specific US sign of solid hydatid is a real challenge for the radiologists.

There have been numerous articles in the medical literature describe the sonographic findings associated with echinococcal cysts[3-12] and they were classified into five types according to the widely accepted imaging classification of Gharbi et al[13]. Barriga et al. were the first to describe solid tumor-like appearance of echinococcal cysts of the liver[17]. But the presence of daughter cysts within the mass was used as a diagnostic and differentiating criterion so the reported cases were not purely solid masses.

Recent reports concentrated on these solid forms of hydatid cyst. Sunsot described the "echo free peritumoral collar" and "echo free spiral" as signs that suggest hydatid disease[18]. These signs correspond to peripheral daughter cysts and fragmented membranes respectively. Another finding that had been published is the presence of posterior acoustic enhancement[19]. Lastly, Francisco et al. described the internal snowstorm echo pattern[16]. This sign represent the innumerable acoustic interface offered by the many membrane fragments and / or minute daughter vesicles. This snowstorm pattern is similar to that described in molar gestation. All these are not specific, can be seen in other hepatic pathologies (e.g. secondaries) and no one of them could be seen in all cases. We have been able to confirm the coiled tramline sign in all of our patients, therefore, it is clearly of great diagnostic value and pathognomonic of solid hepatic hydatid disease differentiating it from other solid hepatic masses. To our knowledge, no other study has been published describing this sonographic sign.

Recommendation

Further large studies including large number of the patient with solid hydatid disease of the liver underwent ultrasonic examination to confirm the diagnosis searching on pathognomonic and diagnostic tram line sign comparing with the final pathological and / or cytological diagnostic report.

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THE EFFECT OF TISSUE PLASMINOGEN ACTIVATOR ON THE MANAGEMENT OF ACUTE MYOCARDIAL INFARCTION

Khalil B. Al-Kubaisi *FRCP*, **Moayed B. Al-Rikabi** *FICMS, CABMS*

Abstract

Background: myocardial infarction is usually the result of occlusive thrombosis in an atherosclerotic coronary artery. Thrombolytic therapy had revolutionized the treatment of acute myocardial infarction: it restores coronary patency, preserves myocardial function, and improves survival.

Objectives: to study the response rate and its relation with patient risk factors, and effect of tissue plasminogen activator, Actilyse, in reducing the in-hospital adverse events, this study was performed.

Patients & Methods: 50 patients received Actilyse within 6 hours from chest pain were enrolled. ST segment reduction of 50% or more in one lead reflecting the infarcted area after 90 minutes of starting Actilyse infusion was regarded as a sign of successful thrombolysis. The effect of patient factors (age, sex, hypertension, diabetes, smoking, and previous ischemic heart disease) on response rate were studied. Patients were followed for the period of in-hospital stay for the development of infarct extension, recurrence of angina, death and complications of thrombolytic therapy.

Results: 16 (32%) of patients showed signs of successful thrombolysis .This varies with the time of chest pain before receiving therapy, the earlier the better. Although statistically not significant:female sex, diabetes mellitus, hypertension, previous ischemia and increasing age, reduce the chance of achieving successful thrombolysis while smoking had no effect. Responders run a better in-hospital course with reduction in post myocardial infarction angina and in-hospital death with no effect on infarct extension. The bleeding complications were one hemorrhagic stroke and one major bleeding with mild bleeding in 12%.

Conclusion: This study shows the importance of the time of starting therapy in determinig response rate. Young age group gets the most benefit. Actilyse reduces post infarct angina and in-hospital death. Infarct extension, late mortality, and effect of patient risk factors on response rate need enlarged study.

Keywords: Actilyse, t- PA, Myocardial Infarction.

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Introduction

ST segment elevation (or left bundle branch block associated) acute myocardial infarction (AMI) is caused by rupture of a coronary artery plaque with super-imposed thrombosis that occlude the coronary artery[1]. One approach to the treatment of thrombosis consists of infusion of thrombolytic agents to dissolve the blood clot and to restore tissue perfusion and oxygenation[2].

Thrombolytic therapy can reduce the risk of in-hospital death by up to 50% when administered within the first hour of the onset of symptoms of AMI. Appropriately employed thrombolytic therapy appears to reduce infarct size, limit left ventricular dysfunction, and reduce the incidence of serious complications such as septal rupture, cardiogenic shock and malignant ventricular arrhythmias[3].

The first generation thrombolytic agents like streptokinase and urokinase, though effective they are not fibrin specific and streptokinase is immunogenic[4]. To overcome some of these problems, the second generation agents like tissue plasminogen activator, t-PA or alteplase were developed, they are more fibrin specific but do produce a mild to moderate decrease in the levels of circulating fibrinogen and plasminogen and prolonged intravenous infusion is the only route[1]. Several third generation thrombolytic agents are now under investigations and many of them were

Dept. Medicine, College of Medicine, Al-Nahrain University.
Address correspondence to Dr. Moayed Bashir.
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approved by the FDA for the treatment of AMI like reteplase. These include mutants of t-PA like monteplase and lantoplase or from bacterial origin like staphylokinase. They are fibrin specific, can be given by bolus doses and clinical trials show promising results[4].

Thrombolytic therapy has been studied more thoroughly than any other in clinical medicine with more than 200000 patients worldwide have participated in myocardial infarction thrombolysis trials (TT)[2]. The gold standard test for assessment of reperfusion was the coronary angiography, but because it is an invasive test and available only in certain centers, other non-invasive clinical tests were studied thoroughly, among these: ECG criteria for successful thrombolysis with sensitivity of about 96% and specificity of 80%[5]. Idioventricular rhythm was also regarded as an indicator of reperfusion[2,3].

Biochemical markers of reperfusion also has been examined in large trials in which elevation of creatinine kinase MB fraction, troponin and myoglobin predict reperfusion with sensitivity and specificity of 80% and 73-100% respectively[5,6].

Aim of the study

To study the response to tissue plasminogen activator in patients with AMI and the effect of cardiovascular risk factors on response and the impact of response on some in-hospital adverse events.

Patients & Methods

The study performed in Al-Kadhimiya Teaching Hospital from July 2001 to November 2001 in the coronary care unit. 50 patients were enrolled with the diagnosis of AMI and thrombolytic therapy started for them with tissue plasminogen activator (Actilyse) after fullfilling the inclusion criteria. The 90 minutes (accelerated or front-loaded) dose regimen used in which 15 mg of Actilyse given as bolus dose, 50 mg as infusion over the first 30 minutes followed by an infusion of 35 mg over 60 minutes until a maximum dose of 100 mg achieved. Aspirin and heparin also were given for all patients.

Inclusion criteria:

1. Symptoms: chest pain, ischemic in character lasting for more than 30 minutes and not relieved by sublingual nitroglycerine.
2. Duration of symptoms less than 6 hours.
3. ECG: standard 12-lead ECG show ST elevation of > 1 mm in two or more limb leads or > 2 mm in chest leads.

At the time of enrollment, data collected by taking history especially of cardiovascular risk factors like hypertension, diabetes mellitus, previous myocardial infarction or angina and smoking. Then the patients were divided into 3 age groups, less than 50 years, 50-60 years and above 60 years. Then they were divided into 3 groups according to the duration of their symptoms, 1-2 hours, 2-4 hours and 4-6 hours, they were divided according to the site of AMI by ECG into anterior and inferior AMI. Reduction in the ST segment elevation of more than 50% within 90 minutes after Actilyse infusion in at least one lead reflecting the infarcted area was considered as a sign of reperfusion.

All the patients were monitored for 90 minutes for the appearance of reperfusion arrhythmias especially idioventricular rhythm and for the final development of pathological Q wave. Then, during the course of in-hospital stay, the patients were followed for the recurrence of chest pain, extension of infarction to leads not previously involved in the initial AMI as well as the development of side effects of Actilyse and these are divided as minor bleeding like bleeding from i.v. line and serious bleeding that needs blood transfusion and hemorrhagic stroke.

Statistical Analysis

The statistical significance of an association between two variables was assessed by chi-square (X^2) test of independence. An estimate was considered statistically significant if its

calculated value was less than α = 0.05 level of significance with 95% confidence.

Results

Of the 50 patients enrolled in the study, with a median age of 58.3 yr (45-65), 38 (76%) were men, and 12 (24%) were women. They have chest pain of a median duration of 3.4 hr. The prevalence of diabetes mellitus, hypertension, previous ischemic heart disease, and smoking among them was 18(36%), 20(40%), 8(16%), and 26(52%) respectively.

16 patients fulfilled the criteria for successful thrombolysis, while idio-ventricular rhythm was recorded in 7 (14%) patients; three of them were in the responder group.

The effect of major cardiovascular risk factors like gender, age, diabetes mellitus, hypertension, history of previous ischemic heart disease, and smoking on the achievement of successful thrombolysis is shown in table 1 which was not significant. The relation between the site of infarction and drug response was not significant also table 2.

The effect of time elapsed between the onset of symptoms of acute myocardial infarction and infusing t-PA on the response was shown in table 3 earlier presentation made reperfusion more likely, although statistically not significant .

Infarct extension was not reduced by successful thrombolysis while post–myocardial infarction angina was reduced but to a level not statistically significant (Tables 4 & 5).

One patient developed hemorrhagic stroke, and another developed major bleeding (haematemesis) and six patients developed minor bleedings. Five patients died during the course of in- hospital admission, four of them were non-responder.

Table 1: The relation between risk factors and drug response

Risk factor	Responding No. (%)	Not responding No. (%)	Total No. (%)	P-value
Gender				
Male Female	14 (37) 2 (17)	24 (36) 10 (83)	38 (100) 12 (100	0.26
Age group				
<50 51-59 > 60	6 (42.9) 4 (33) 6 (25)	8 (57.1) 8 (67) 18 (75)	14 (100) 12 (100) 24 (100)	>0.5
Diabetes				
Positive Negative	4 (22) 12 (37)	14 (78) 20 (63)	18 (100) 32 (100)	0.37
Hypertension				
Positive Negative	6 (30) 10 (33)	14 (70) 20 (67)	20 (100) 30 (100)	>0.5
History of previous ischaemic heart disease				
Positive Negative	2 (25) 14 (33)	6 (75) 28 (67)	8 (100) 42 (100)	>0.5
Smoking				
Smoker Non smoker	9 (34.7) 7 (29.1)	17 (65.3) 17 (70.9)	26 (100) 24 (100)	0.21

Table 2: The relation between the site of infarct and drug response

Site	Responding No (%)	Not responding No (%)	Total No (%)	P-value
Anterior Inferior	7 (33) 8 (31)	16 (67) 18 (69)	24 (100) 26 (100)	> 0.5

Table 3: The relation between chest pain and drug response

Duration of symptoms	Responding No (%)	Not responding No (%)	Total No (%)	P-value
<2 hours	7 (44)	9 (56)	16 (100)	0.25
2-4 hours	4 (32)	11 (68)	16 (100)	
4-6 hours	4 (32)	14 (77)	18 (100)	

Table 4: The relation between infarct extension and drug response

Infarct extension	Responding No (%)	Not responding No (%)	Total No (%)	P-value
Positive	2 (33)	4 (67)	6 (100)	> 0.5
Negative	14 (32)	30 (18)	44 (100)	

Table 5: The relation between postinfarct angina and drug response

Angina	Responding No (%)	Not responding No (%)	Total No (%)	P-value
Positive	2 (17)	10 (83)	12 (100)	0.26
Negative	14 (37)	24 (63)	38 (100)	

Discussion

Successful reperfusion was seen in 32% of patients, this is lower than rates reported by other studies and this can be explained by delayed presentation of our patients[7,8]. Idioventricular rhythm was seen in 18.7% of responders and in 11.7% of non-responders so it has no significant value as a marker of reperfusion, this is consistent with the previous studies[3,7,8]. The chance of achieving successful reperfusion was decreased with increasing age and this is also consistent with other studies[7,8].

Males show higher rate of reperfusion than females. Age effect is partly responsible since females usually affected by atherosclerosis an average of 10 years later than males and this is consistent with other studies[1-3].

Hypertension had no significant effect on reperfusion. Previous studies showed conflicting results[7,8]. Diabetic patients showed reduced response to thrombolytic therapy. This association, though statistically insignificant, is consistent with other studies since diabetic patients have more advanced atherosclerosis and tend to present later than non-diabetics[7,8]. Smokers have better chance of achieving successful response. Gender effect is partly responsible since most of the smokers (88%) were males.

The presence of previous ischemic heart disease reduces the chance of achieving successful thrombolysis since patients with ischemic heart disease tend to be older than those presented with AMI for the first time, have more than one risk factor for failure of thrombolysis like diabetes and hypertension[7,8].

In all studies conducted in the field of thrombolysis, time is of central importance, this was clearly shown in this study where the rate of successful reperfusion was 43.7% in the 1-2 hours group and decreased to reach 22.2% in the group received thrombolytic therapy 4-6 hours after starting their symptoms[1,7,8].

The site of AMI has no effect on response and this is also consistent with most of previous studies[7,8]. Q wave appears finally in all but 4% of patients and extension of infarction occur in similar proportion in both responders and non-responders; this may be due to small number of cases included in this study since other studies leaves no doubt that infarct extension is markedly reduced by

thrombolytic therapy[9]. The overall recurrence of chest pain was similar to that reported by other studies but in contrast to other studies there were insignificant reduction in the recurrence of chest pain in the responder group as compared to non-responders.

There were single case of intracranial hemorrhage and one other major bleeding (hematemesis that necessitate blood transfusion) and minor bleeding was seen in 12% of patients but it was very easily controlled.

Five patients died during the course of in-hospital admission, 4 of them were non-responders in whom the cause of death was cardiogenic shock and one in the responders group died with hemorrhagic stroke. Although the impact of thrombolytic therapy on mortality was similar to other studies, the effect on such an important parameter (death) cannot be guaranteed by such a relatively small number of patients.

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TEACHING CARDIC PATIENTS HOW TO MANAGE THEIR MEDICATIONS

Nabil A. Antowan[1] *DCH PhD*, **Amar T. Al-Hamdi**[2] *FRCP*, **Hakema S. Hassan**[3] *MSC*

Abstract

objective: to help patients acquire and retain sufficient information about their cardiac drugs for which they have been teaching in hospital and had to achieve an adequate self care regarding their drugs after leaving the hospital.

Methods: Using pre-post tests approach.

Results: the sample consists of 60 patients with Heart disease. The results revealed increase in the depth of knowledge, understanding of the patients to the names and action of the drugs, and when they would need their Dr.

Conclusion: Cardiac teaching program was effective increase in Knowledge about drugs therapy in the study group. According to the results the investigators recommend expansion of the program to include with other disease and drugs.

Key words: Cardiac rehabilitation, teaching patients and cardiac drugs.

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Introduction

Patients are usually sent home with medication that require specific knowledge for safe administration[1,2]. Nurses spend much time teaching patient about current treatment modalities, and what to expect after discharge from the hospital[3].

From observation in out-patient clinic at Al-Kadhimiya Teaching Hospital, we suspected that patients knew little about their medications. Some patients who run out of digitalis or potassium chloride before a visit did not have prescriptions refilled. Other patients attempted to regulate their diuretic therapy without any apparent understanding of the purpose and effect of those drugs.

The objective of our study was to help patients acquire and retain sufficient information about their cardiac drugs for which they have been taught in hospital and had to achieve an adequate self care regarding their drugs after leaving the hospital.

Patients & Methods

A quesi experimental design using pre-post tests approach, the study population consisted of 60 patients divided into the study group (30 patients) and other control group (30 patients) (non-probability sample), consecutively admitted to the coronary care unit in Al-Kadhimiya Teaching Hospital during April, May and June 2001. The following specific criteria considered in the study:

1. First attack of myocardial infraction (MI), unstable angina and heart failure (H.F).
2. Patients who have willingness to participate in this study.
3. Adult from age 30-70 years old.
4. Families are encouraged but are not required to participate in this study.
5. The permissions of their attending cardiologist.

The patients who were excluded from this study are those who were unable to communicate Arabic, disoriented or with speech impairment and those who were too ill to complete the interview.

An educational program was carried out by the investigator who provided pre and post tests. The pre test was set to find out how much the patients actually know about their medications. Post test was done before discharge from the hospital. The test score was either wrong or

[1] Dept. Clinical Physiology, [2] Dept. Medicine (College of Medicine, Al-Nahrain University), [3] Dept. Medical-Surgical Nursing (Nursing College, Baghdad University).

Address correspondence to Dr. Nabil A. Antowan, e-mail: nabil_anto@yahoo.com

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correct, the total marks of the test were out of 100 and the patients who scored less than 60 marks in the pre test were included to participate in the study. Time for each pre and post test was 15 minutes.

Preparation of the educational program was through reviewing the related literature by using interviewing format prepared specially for this purpose and sending the educational program to 5 experts to take their scientific opinions: The program includes the following items:

1. Names of the drugs were announced loudly and after that the patients repeated it.
2. A sample was taken out from the bottle and the patients looked at it and felt it.
3. The action of the drugs was described.
4. The dosage and frequency were discussed using a drug calendar which was arranged by investigators.
5. Possible unwanted effects were described including the indications for notifying doctor about it.
6. Weather there is anything special about specific drugs, for example not taking Aspirin dosage on empty stomach or taking diuretic dosage at the proper time which suits the patients.

Once the patient examined by their cardiologist and their condition was stabilized, providing they had fulfilled with the above criteria, they were interviewed and information about their conditions was taken. Both groups were either on one or combinations of the following drugs: anticoagulant, digitalis preparation or diuretic or antianginal therapy. Most of the patients were discharged on third to sixth days after admission to the coronary care unit.

The individual discussion in the study group was holding on a daily basis with an investigator who spent 2 sessions per day in general, duration for each session 20 minutes. Patients in the control group were allowed to learn about their disease in whatever manner was presently being used at the institution in which they were hospitalized. If the patients in the control group asked the investigator questions, they were instructed to refer their questions to appropriate member of the health team, e.g nurse, and physicians.

Validity of knowledge test: An investigator at one of the study institution was able to validate that the content of the knowledge test question and the objective of the cardiac teaching program. Reliability coefficient was 0.78 that was statistically acceptable.

Statistical Analysis

Percentage was used to calculate the description of the sample. Mean, stander deviation and t-test value were computed to estimate the differences between pre and post tests for two groups. Kolmogrove-smirnove used two samples to determine the significant differences between a pre and post test for control and study group.

Results

The total sample included 60 patients, 30 patients in the study group (7 female, 23 male) were age ranged between 31-70 years (mean age 54 years). 20 patients in the study group were diagnosis of AMI, 5 patients with unstable angina, 4 patients with AMI and HF and one patient with HF alone. 30 patients in the control group (9 female, 21 male) were age ranged between 31-70 years (mean of age 55). 17 patients in the control group were diagnosis of AMI, 9 patients with unstable angina, only one patient with AMI and HF and three patients with HF.

Formal education of the patients was as follows: in study group, the majority was illiterate (15 patients) and the rest either read and write primary, intermediate, secondary school or institute gradual.

In control group, the majority were illiterate (12 patients) and the rest either read and write, primary, intermediate, secondary school or institute gradual (Table 1).

The results in this study showed that in the study group 2 patients in a pre test 14 patients in a post test knew the name of groups 4 patients in a pre test, and 21

patients in a post test knew the description the action of drugs, 18 patients in a pre test and 30 patients in a post test knew they would take. their medicine and how much, 3 patients in a pre test 27 patients in a post test knew when they would need their DR. 3 patients in a pre test and 28 patients in a post test knew if there is anything special they need to do, 29 patients in a pre test and 30 patents a post test knew general description of medicine.

In the control group, the results of the a pre and post tests were the same in a pre and a post tests, 9 patients knew the name of drugs, in a pre and a post tests, 6 patients knew the description the action of the drugs, in a pre and a post tests 29 patients knew when they would take medicine and how much , in a pre and a post tests 4 patients knew they would need their doctor in a pre and a post tests 1 patient knew if there is anything special they need to do and in a pre and a post tests 30 patients knew general description of medicine (Table 2).

The comparison of the post test results in regard to name of drugs, description the action of the drugs, when they would need their Dr, if there is anything special they need to do. Significantly different in the experimental group were than control group $p < 0.05$. Where as comparison of the post test results in regard to when they would take their medicine and general description of medicine was no significant difference ($p > 0.05$) was found between the study group and control group (Table 2).

Finally the comparison of the pre and post test scores between the study and control group showed that the study group gained more knowledge ($p < 0.05$) than the control group (Table 3).

Table 1: Characteristics of the Sample

Variables	Control group		Study group	
	Number	* %	Number	%
Age				
31-40	6	20	1	3
41-50	6	20	13	44
51-60	6	20	10	33
61-70	12	40	6	20
Mean Age	55		54	
Sex				
Male	21	70	23	77
Female	9	30	7	23
Diagnostic Cases				
AMI	17	57	20	67
Unstable angina	9	30	5	17
HF	3	10	1	3
MI & HF	1	3	4	13
Level of Education				
illiterate	12	40	15	50
Read and Write	6	20	4	13
Primary	4	13	5	17
Intermediate	2	7	3	10
Secondary	3	10	2	7
Institute	1	3	1	3
University	2	7		

* % = Percentage of patients.

Table 3: Comparative difference pre and post tests between control and study groups

Knowledge test	N= 30 control group		N=30 Study Group			*C.S	
	X	SD	X	SD	T value	d.f.	C.S.
Pre-test	12.16	11.6	9.33	10.15	1.0056	58	ns
Post-test	12.16	11.6	25	6.32	2.421	58	s

C.S: Comparative difference in pre and post tests between Study and control groups, S: Significant difference at p<0.05, N.S: No Significant difference at p < 0.05, d.f.: Degree of freedom

Table 2: Comparison evaluation of pre and post tests in patients with heart disease between control and Study groups

Items	control group			Study group		C.S			
	pre test No.	post test No.	C.S control prexpo.	pre test No.	post test No.	C.S Exp. Prexpo.	KS & P value	KS control Exp.	KSP Value
Name of drugs	9	9	Ns	2	14	S	0.000	Ns	0.191
Described the action of drugs	6	6	Ns	4	21	S	1.000	S	0.318
When would they take their medicine & how much	29	29	Ns	18	30	Ns	0.000	Ns	0.024
When would they need their doctor	4	4	Ns	3	27	S	0.000	S	0.461
Is there anything special they need to do	1	1	Ns	3	28	S	0.000	S	0.598
General description of medicine	30	30	Ns	26	30	ns	0.125	NS	0.017

* C.S: Conparative significant between pre and post tests in Study and control groups, S: Significant difference at p < 0.05, N.S: No significant difference at p > 0.05, K. S.: Kilmogerive smirnove.

Discussion

It's apparent that learning about the medical regimen is fundamental in patient own care[4]. Teaching program on medication to be a part of the overall hospital cardiac rehabilitation program[5].

This study demonstrated that the patients are capable of learning during the early stages of recovery about their drugs. The results of post test are significantly different than pre test, in study group in regard to name of drugs, description the action of the drugs, when they would need their Dr and if there is anything special need to do.

Patients continue to gain knowledge after series of discussion involved in cardiac rehabilitation program. It has been shown that the education of the patient positively influence the behavior of the patient on drugs therapy[6,7].

The experimental group patient gained significant more knowledge than the control group (p < 0.05). This indicated that the effectiveness of rehabilitation instruction led to improve in knowledge of patients following educational program.

Patient who received instruction in unstructured manner about how to care for themselves will not achieve a high score on paper and pencil test as these patients who will received instructions in an organized structures manner[4,8-10].

Patient education has been assessed and recognized by the nursing profession as an important nursing function forming an integral part of daily patient care. However, there are a number of barriers which effect patient education such as frustration which may result from learning the patient who do not remember what they have been taught in hospital, who not ready to hear and retain teaching

during that period. Nurses need to understand the briers of cardiac patients within the phases of recovery and acquire shills for accurate assessment of patient's readiness to learn during the early stages of recovery.

In learning program of our study .Two areas of greatest difficulty was shown one is the name of drugs and the other is the action of drugs but we observed that patient's degree of motivation appeared particularly important in achieving improvement in this program. Three patients who were illiterate and seemed eager to learn rehabilitation did not do during rehabilitation program. The presence of family members during teaching sessions appeared to be beneficial to motivates and reinforce our teaching. A nurse educator would be better than outside researcher to select a proper time to educate the patient about his medication.

It was concluded that cardiac teaching program was effective in knowledge about drugs therapy in the study group. Based on the results the investigators recommended that a family member or close friend or neighbor may be taught and should be expanded to include self administration of drugs therapy by the patient several days prior to discharge.

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EFFECT OF CALCITONIN NASAL SPRAY IN PREVENTION OF BONE FRACTURES IN POSTMENOPAUSAL WOMEN

Adnan Anoze *MRCP*

Abstract

Background: Osteoporosis a condition, which can lead to fractures, deformity, pain and disability. Patients with low bone mineral density have a high risk of fracture and should actively consider for treatment to reduce their risk.

Objectives: This study was to determine the effect of calcitonin nasal spray (miacalcic) therapy in prevention of fractures in postmenopausal osteoporosis.

Methods: Patients were randomly assigned to treatment with calcitonin nasal spray (100 U/day for 2 years). The study enrolled 320 women aged 52 - 71 years with low bone mass, all patients had no history of fractures and x- ray of hips, spine and wrists showed no evidence of fractures.
One hundred sixty women received calcitonin (100 U/day) as nasal spray with calcium carbonate tab (one gram /day) in a cyclical way and the other 160 women received only calcium carbonate (one gram /day). All women were followed for two years.

Results: Twenty two patients (13.7 %) of those who did not receive calcitonin developed fractures of the vertebrae, neck of the femur or the wrist joint while only one patient (0.6 %) who received calcitonin developed collapse of a vertebra.

Conclusion: Osteoporosis is a condition, which can lead to fractures of bones in postmenopausal women. Prevention of bone loss appears to be the most effective means of reducing the incidence of fragility fracture achieved by the use of calcitonin nasal spray which lead to increase in bone mass over the first 1 to two years.

Keywords: Calcitonin nasal spray and bone fractures

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Introduction

The world health organization (WHO) accepted the definition of osteoporosis as a disease characterized by low bone mass and microarchitectorial deterioration of bone tissue leading to enhanced bone fragility and a consequent increased risk of fracture[1].

Menopause has an increased risk of low bone mass and fragility fractures. Fracture risk rises soon after menopause[2,3].

The number of women living in the world who are 50 years old or older has been estimated and found to be increasing. One in three women probability has osteoporosis, a condition which can lead to fractures, deformity, pain and disability[4,5]. With time osteoporosis is becoming an important and costly disorder that is rapidly increasing in prevalence in our society.

The number of patients becoming bed ridden due to osteoporosis related fracture has been socially important health issue[6].

Recently the introduction of rapidly action bone absorption suppressant with a clear mechanism accelerated the development of osteoporosis treatment, calcitonin, Bisphosphanates and hormone replacement therapy after a therapeutic option for preventing and treating osteoporosis[7].

For many reasons the menopausal women refuse the hormonal replacement therapy because they do not like the return of menstruation in addition to other side effects of the hormones[8], the finding of a substitute is important.

Here we assess the calcitonin nasal spray effect in preventing the development of fractures in post menopausal women.

Dept. Medicine, College of Medicine, Al-Nahrain University.
Address Correspondence to Dr. Adnan Anoze
E.mail : anoze4a@yahoo.com
Received 18th January 2004: Accepted 22nd June 2004.

Methods

Three hundred twenty postmenopausal women aged 52-71 year with an average of (61 year) were enrolled in this study. The cases were regular attendees of the out patient clinic in Al-Kadhimiya Teaching Hospital. The study was performed from September 2000 to September 2003.

All patients had no evidence of recent development of fracture but only osteopenia seen by radiological study. All other cases of osteoporosis had been excluded by history taking, physical examinations and investigations including hormonal study especially thyroid function test.

All cases had no history of steroid consumption and they had no history of any disease which might need steroid as treatment. No one of the cases has been given any hormonal replacement therapy for menopause.

The patients has been divided into two equal divisions 160 for each group all of them received calcium supplement in a form of calcium carbonate one gram per day by the oral rout. The patients followed monthly with a full history and examination in each visit.

Radiological examinations (thoracic spine, lumbosacral spine, hips and wrist) have been done for every patient. Assessment of bone mineral density (BMO) was done on visually analyzed radiograph.

The patient was judged as having a vertebral fracture if she developed at least 4mm decrease in height in comparison to any other vertebral body. One hundred sixty patients received calcitonin nasal spray therapy (100U/puff) and 160 patients kept on calcium carbonate tablets (one gram per day). All patients were followed in the department of rheumatology in the university hospital.

Results

Table 1 showed that five patients (3%) of those who did not receive calcitonin developed fractures of the hips in the first year and the number increased to 8 patients (5%) in the second year and 16 patients (10%) developed vertebral fractures in the first year and the number of patients who developed vertebral fractures increased to 28 patients (17.5%) during the second year.

While those who received calcitonin 3 patients (1.9%) developed fracture of the hips in the first year and only 2 patient 1.25% in the second year which showed a significant deference, $P<0.05$. Also for the vertebral fractures the non-calcitonin taking group 16 patients (10%) develop fracture of the vertebrae in the first year and 28 patients (17.5%) in the second year in comparison to 8 patients (5%) in the first year and 6 patients (3.75%) in the second year for those who received calcitonin nasal spray as treatment ($P<0.004$). No one of the patients developed Colles fracture during the first and the second year.

Table 1: Comparison between the patients who received calcitonin with those who did not receive calcitonin after two years of treatment

Group two calcium carbonate plus calcitonin nasal spray			Group one calcium carbonate only	
2nd year	1st year	2nd year	1st year	Fracture
Hip	5 patients (3%)	2 patients (1.25%)	3 patients (1.9%)	8 patients (5%)
Colles	1 patient (0.6%)	Nil	Nil	2 patients (1.25%)
Vertebral	16 patients (10%)	6 patients 3.75%	8 patients (5%)	28 patients (17.5%)

Discussion

Prevention of bone loss appears to be the most effective means of reducing the incidence of fragility. For pharmacological intervention the identification of individuals at risk of fractures will help to restrict therapy to those who need it. Calcitonin is approved for the treatment of postmenopausal osteoporosis.

Davidson et al mentioned (in osteoporosis the mortality and morbidity after fractures are high especially hip fracture, more attention to hip fracture prevention is needed)[9].

Reports from many countries have shown an increasing use of calcitonin nasal spray in the treatment of osteoporosis on the assumption of calcitonin prevents bone loss in the hip and spine in postmenopausal women[10].

Jaraid M.K and Cooper said (agents such as calcitonin, PTH and fluorides are of less certain benefit in preventing fracture)[11].

In our study there was clear reduction in the number of fractures after the use of calcitonin nasal spray during the first two years and there is very clear evidence from the number of patient who developed fractures of hips and vertebrae. The rate of hip fracture was 3% in the first year increase to 5% in the second year in those who did not receive calcitonin while in the group of patients who received calcitonin the figure was 1.9% in the first year decreased to 1.25% in the second year. Our findings where in agreement with WO-T and Adachi T.D. who motioned that calcitonin has demonstrated the ability to reduce vertebral and hip fracture rate with minimal change in bone density[12,13].

Conclusion

1. calcitonin nasal spray is a good drug for prevention of fracture in postmenopausal osteoporosis.
2. It may be possible to obtain continued increase in bone mass if calcitonin is administered cyclically.

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POST-STROKE SEIZURE(S)

A.M. Abdul-Kareem[1] *FRCP*, **HASAN A. Al-Hamdani**[1] *FICMS*,
Akeel J. Al-Bahadli[2] *FICMS*

Abstract

Background: Cerebrovascular disease is an important cause of epilepsy, particularly in elderly people. Many facts point to an etiological connection between the seizures and the underlying arterial disease. The frequency rate of cerebrovascular etiology is significantly higher in studies carried out in developed world.

Objective: To evaluate the patients with seizure(s) after stroke to clarified the connection between the seizures and the etiological underlying vascular disease

Patients & Methods: From March 2000 to March 2002, 58 patients with seizure after stroke were referred to the neurologic department of the Teaching hospital of Iraqi Medical College. The study is cross sectional prospective. The clinical diagnosis of stroke was reserved for the nature (ischemic or hemorrhagic), cortical involvement and side (left or right cerebral hemisphere).Among these patients, seizure(s) identified, evaluated and classified. Statistical analysis was done using SPSS (statistical package for social sciences) version 7.5.

Results: The study includes patients with post stroke seizure(s) with no history of seizure before the onset of the stroke. 20 (34.5%) females and 38 (65.5%) male form a cohort of patients over 2 years. Patients of all ages were included 18 (31.1%) were under 50 years, 40 (68.9%) above 50 years. A total of 41(70.7%) patients had cerebral infarction, 17 (29.3%) had intracerebral haemorrhages.

The onset was significantly later in older age group (above 50) years **(**P Mann-Whitney = 0.003). The relative frequency of cortical location was significantly higher among subjects with infarction (95.1%) than those with haemorrhage (29.4%) **(**P (χ^2) < 0.001).The onset was significantly delayed in those with cortical affection compared to those with subcortical (P Mann-Whitney = 0.003). Gender and level of central nervous system insult had no important bearing or statistical significance to the occurrence of seizures. Patients with infarction in the older age group above 50 years had a significantly higher risk of having seizure (P (χ^2) = 0.28[NS]).

Conclusion: Patients with infarction stroke in the age group above 50 years had a significantly higher risk of developing seizure(s) after stroke.

The risk of having late onset seizure increases significantly in the age group above 50 years old and infarction as the cause of central nervous system insult.

The onset of seizure was significantly delayed in those with cortical affection compared to those with subcortical.

Key worlds: stroke, seizure and epilepsy

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Introduction

Cerebrovascular disease is an important cause of epilepsy, particularly in elderly people. When seizures complicate a clinical stroke they have a devastating effect on morale and further impair the already compromised quality of life[1]. The reported incidence of seizure after stroke varies from 4 to15%.The incidence of epilepsy after stroke ranges from 4 to 9%. 67% of patients with seizure after stroke had focal seizures with or without secondary generalization[2]. The frequency rate of cerebrovascular etiology is significantly higher in studies carried out in the developed world[3]. In Saudi Arabia, The epilepsy was symptomatic in 32% of the cases; stroke is the cause in 1%[4].

Most of the studies published relate to cortical infarction, subarachriod and intracranial hemorrhage[5]. Subcortical infarcts have been associated with post-stroke epileptic seizures, although less

[1] **Section of Neurology,** [2] **Section of Nephrology, Dept. Medicine, College of Medicine, Al-Nahrain University.**
Address Correspondence to Dr. Hasan A. Al-Hamdani.
Received 19th January 2004: Accepted 22nd June 2004.

frequently than cortical ischemia[6]. Reports of seizures complicating basal ganglia hemorrhage and lacunar infarction suggest that the association between post stroke seizure(s) and cortical damage may not be as exclusive as previously thought[1].

Two types of seizure(s) were defined: 'early-onset' seizures (occurring within the 14 days following the stroke) and 'late-onset' ones (after the 14th day)[7].

Many facts point to an etiological connection between the seizures and the underlying cerebral arterial disease particularly the coincidence of laterality between the involved hemisphere and the affected artery (in the case of focal seizures) and the time relation between the onset of typical features of cerebral insult and the occurrence of seizure(s). Indirect evidence can be added, such as the exclusion of other possible causes of epilepsy and the overall incidence of seizures in both groups of patients, which is definitely higher than that of epilepsy in an age-matched general population. In view of all these considerations, the hypotheses of a coincidental association or of a questionable relationship seem hardly tenable[8].

The pathophysiology of poststroke seizures is not fully elucidated; probably it is different for early and late seizures. Several investigations evaluated the connection between seizures and site of the vascular insult; the results are not consistent[5]. The most common type of seizures is simple focal seizures[5]. Actors that appear to be predictive of seizure development were the presence of large cortical infarcts and the presence of apparently preserved cerebral tissue within the infarcted area[9].

Patients & Methods

From March 2000 to March 2002, 58 patients with seizure were identified in the department of neurology of Al-Kadhimiya Teaching Hospital with seizure(s) after stroke admitted to the hospital or visited the neurology consultation clinic within a period of two years. Using a total of these patients has post-stroke first-time seizure(s). The study is cross sectional prospective and consecutive with modifications given below. We proposed to analyze these features in a consecutive series of patients. Included in the study were stroke patients referred to our department because of seizure(s) with history of stroke or proved to be caused by cerebral vascular insult or diagnosed as stroke presented with seizure. Seizures types were typified according to the repot of the ILAE task force on classification and terminology 2001[11].

All patients with cerebral infarction could be classified into subtypes according to their clinical presentation and neuroimaging (CT and MRI) study. Each patient with cortical involvement labeled as cortical even if there is subcortical involvement with it.

Onset seizure was defined as occurring with 24 hours as the onset of stroke. Seizures occurring later were classified as early-onset seizures (occurring within the 14 days following the stroke) and late-onset ones (after the 14th day) included from the study.

The patients underwent a full neurologic examination on admission and were investigated to confirm the diagnosis and to exclude other possibilities for seizures.

The clinical and laboratory investigations included a detailed history, a general and neurological clinical examination, evaluation of the main blood and urine parameters, brain neuroimaging studies ECG, standard 16 channels EEG recording. In several patients further investigations carried out, coloured dopplerrs ultrasonography and echocardiography in patients with infarction and cerebral angiography in patients with intracranial haemorrhage and carotid angiography in patient with evidence of carotid artery stenosis. The clinical diagnosis of stroke was reserved for the nature of stroke (ischemic or hemorrhagic), cortical involvement and side (left or right cerebral hemisphere) was determined.Among those patients,

seizures identified and evaluated; we analyzed the type and characteristics of the seizure(s).

A clinical diagnosis of epileptic seizure was reserved for the following condition:

1. The patient had focal seizure and their subtypes.
2. The patient had focal seizure followed by secondary generalization.
3. The patient have generalized convulsion without focal onset.
4. The patient have continuous seizure types (Generalized status epilepticus or Focal status epilepticus)

Excluded from the study were:

1. Patients with history of seizure, before the onset of the stroke.
2. Patients with neurological or systematic illnesses that can provoke seizures. Patients with compromised function of the heart, kidneys, lungs or liver and patient with severe disabling disease such as multiple sclerosis and advanced malignant disease were excluded.

Statistical analysis was done using SPSS vr7.5 (statistical package for social sciences). An expert statistical advice was sought for. The statistical significance of association between 2 categorical variables was assessed by chi-square test (fissure exact test and likelihood ration significance tests were used when the conditions for a valid chi-square test was not met). Mann-Whitney non parametric tests were used to assess the statistical significance of difference median time of onset of seizure between 2 study groups. Multiple logistic regression models were used to study the net effect of each of four explanatory variables on the risk of having late onset seizure. P value, less than 0.05 level of significance was considered statistically significant.

Results

The research includes 58 patients with a post stroke seizure(s) with no history of seizure before the onset of the stroke, 20 (34.5%) females, and 38 (65.5%) males, from a study of cohort of patient over 2 years. Patients of all ages were included: 18 (31.1%) were under 50 years, 40 (68.9%) above 50 years. A total of 41(70.7%) patients had cerebral infarction, 17 (29.3%) intracerebral haemorrhages. Cortical involvement was found in 44(75.9%) patients and 14 (24.1%) patients had subcortical lesions (Table1).

Infarction as a cause of central nervous system insult significantly increases the risk of having late onset seizure compared to haemorrhagic central nervous system insult (after controlling for the effect of age) (Table 3).

The onset was significantly later in older age group (above 50) years old (Table2). The relative frequency of cortical location was significantly higher among subjects with infarction (95%) than those with haemorrhage (29.4%) (Table 4). The onset was significantly delayed in those with cortical affection compared to those with subcortical (Table 4). Gender and level of central nervous system insult had no statistically significant (Table 6).

Table 1: Frequency distribution of the study sample by certain variables

Variables		Number	%
Age in years	<30	7	12.1
	30-49	11	19
	50-69	13	56.9
	70+	7	12.1
Gender	Female	20	34.5
	Male	38	65.5
Hemisphere affected	Left	31	53.4
	Right	27	46.6

Table 1: *Continued*

Variables		Number	%
Type of stroke	Hemorrhage	17	29.3
	Infarction	41	70.7
Level of stroke	Subcortical	14	24.1
	Cortical	44	75.9
Type of seizure	Focal +/- secondary generalised	31	53.4
	Generalised	24	41.4
	Focal status epilepticus	3	5.2
Onset of seizure relative to stroke incidence	Immediately with (onset)	15	25.9
	Early	20	34.5
	Late	23	39.7
	Total	**58**	**100**

Table 2: The relative frequency of different characteristics by age

Variable	Subtype	P value	Age in years			
			<50		>50	
			Number	%	Number	%
Type of stroke	Hemorrhage	**P (χ^2) = 0.28*[NS]***	7	38.9	10	25
	Infarction		11	61.1	30	27
Level of stroke	Subcortical	**P (Fisher's exact) = 0.22*[NS]***	6	33.3	8	20
	Cortical		12	66.7	32	80
Type of seizure	Focal +/- secondary generalised	**P (Likelihood ratio) = 0.25*[NS]***	9	50	22	55
	Generalised		9	50	15	37.5
	Focal status epilepticus		0	0	3	7.5
Onset of seizure relative to stroke incidence	Immediately with (onset)	**P Mann-Whitney = 0.003**	9	50	6	15
	Early		6	33.3	14	35
	Late		3	16.7	20	50
	Total		**18**	**100**	**40**	**100**

Table 3: The association between different characteristics and type of stroke whether haemorrhagic or infarction

Variable	Subtype	P value	Type of stroke			
			Hemorrhage		Infarction	
			Number	%	Number	%
Level of stroke	Subcortical	P (χ^2) < 0.001	12	70.6	2	4.9
	Cortical		5	29.4	39	95.1
Type of seizure	Focal +/- secondary generalised	P (Likelihood ratio) = 0.32*[NS]*	9	52.9	22	53.7
	Generalised		8	47.1	16	39
	Focal status epilepticus		0	0	3	7.3
Onset of seizure relative to stroke incidence	Immediately with (onset)	P Mann-Whitney = 0.02	5	29.4	10	24.4
	Early		11	64.7	9	22
	Late		1	5.9	22	53.7
		Total	**17**	**100**	**41**	**100**

Table 4: The association between different characteristics and level of central nervous system insult whether cortical or subcortical

Variable	Subtype	P value	Level of stroke			
			Subcortical		Cortical	
			Number	%	Number	%
Type of seizure	Focal +/- secondary generalised	P (Likelihood ratio) = 0.42[NS]	8	57.1	23	52.3
	Generalised		6	42.9	18	40.9
	Focal status epilepticus		0	0	3	6.8
Onset of seizure relative to stroke incidence	Immediately with (onset)	P Mann-Whitney = 0.007	6	42.9	9	20.5
	Early		7	50	13	29.5
	Late		1	7.1	22	50
Total			**14**	**100**	**44**	**100**

Table 5: The association between timing of seizure onset relative to stroke incidence and clinical type of seizure

Onset of seizure relative to stroke incidence	Type of seizure					
	Focal +/- secondary generalised		Generalised		Focal status epilepticus	
	No.	%	No.	%	No.	%
Immediately with (onset)	11	35.5	2	8.3	2	66.7
Early	6	19.4	13	54.2	1	33.3
Late	14	45.2	9	37.5	0	0
Total	**31**	**100**	**24**	**100**	**3**	**100**

P (Kruskal-Wallis) = 0.16[NS]

Table 6: Multiple logistic regression model with the risk of having a late onset seizure as the dependent variable and 4 independent variables

Independent variable	OR	P value
➔ Infarction (compared to haemorrhagic central nervous system insult)	18.5	0.007
➔ Age above 50 years (compared to younger age group)	5	0.03

- **Gender and level of central nervous system insult had no important or statistically significant adjusted OR when included in the model.**
- **P (model) < 0.001**
- **Accuracy of predicted classification 72.4 %.**

In a multivariate analysis, patients with infarction in the age group above 50 years old had a significantly higher risk of suffering from seizure after stroke

A multiple logistic regression model was used to assess the risk of having a late onset seizure according to the status of 4 independent (explanatory) variables these were gender, level of central nervous system insult, age and type of central nervous system insult (hemorrhagic versus infarction). Gender and level of central nervous system insult had no bearing or statistical significant impact on the risk of having later onset seizure when included on the model.

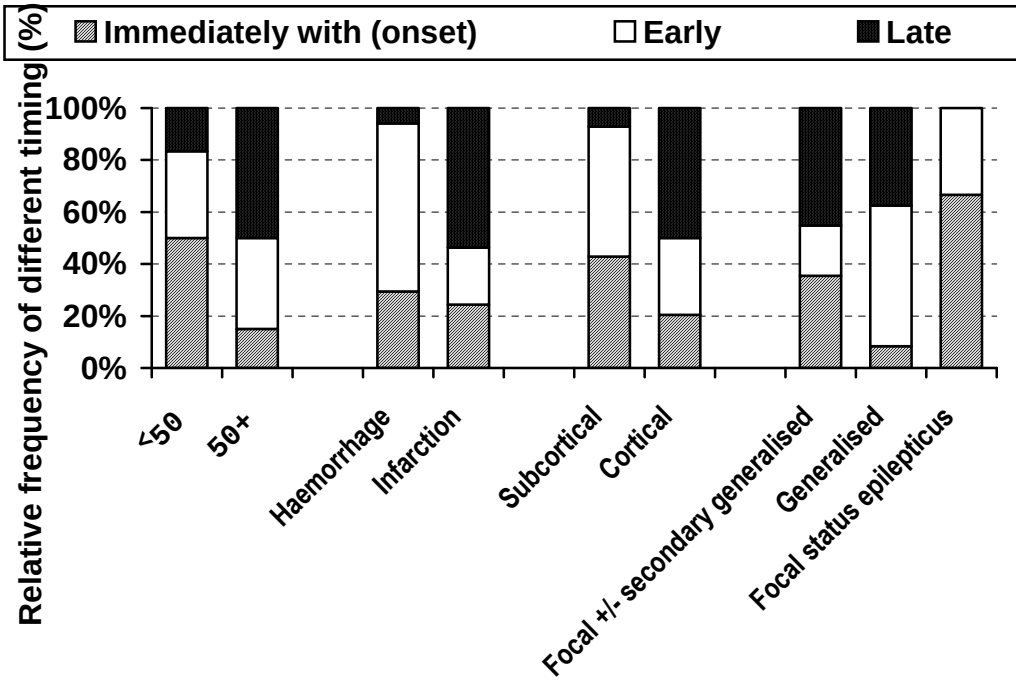

Figure 1: Stacked bar chart showing the relative frequency of 3 categories of seizure timing by different independent variables

Discussion

Though there are many reports on post stroke seizures there is still much controversy about them. The studies of seizures after stroke have largely been retrospective or prospective studies of stroke patients to recognize the incidence and the recurrence of seizure and epilepsy after and the onset of stroke. In this study, we tried to evaluate the patients with seizure(s) attributed to vascular insult. The patients were viewed depending on age and gender of the patients, moment of seizure(s) onset, seizures type, stroke type (ischemic or hemorrhagic stroke), and cortical involvement to evaluate each seizure type with age and other variables. Hemorrhagic stroke is (29.3%) and infarction (70%). The distribution of seizure type show that focal with or without secondary generalization is (53.5%), focal status epilepticus in (5.2%) and Generalized in (41.4%). Higher incidence of generalized seizure without focal element in our study (Table 1) in comparison to that of Glawar et al[2] may be because of the focal fits is mild that can be missed by the patient especially non motor focal seizures.

Cortical involvement is present in (75.9%).95.1% of the ischemic strokes this study is cortical and 29.4% of the hemorrhagic one (Table 3). The study shows that, all types of seizures are more with cortical stroke in the two types of post stroke seizure(s); early onset seizures and late onset ones (Table 4). This finding can be explained by epileptogenic focus formation in cortical involvement of the stroke, as seizures are primarily a cerebral grey matter manifestation.

The onset was significantly delayed in those with cortical affection compared to those with subcortical (Table 4). No significant difference in cortical and subcortical stroke in the early onset seizure but infarction as a cause of central nervous system insult significantly increase the risk of having late onset seizure (Table 3). Patients with infarction in the age group above 50 years old had a significantly higher risk of suffering from seizure(s) after stroke (Table 6). This is explained by the high incidence of cortical

involvement in the ischemic stroke (Table 3).

Hemorrhage has a high incidence in this study (29.3%) in compared to the general incidence of hemorrhage among stroke patients, most probably because hemorrhage is a major event the patients usually complain early and the seizure easily related to it but with ischemia may be mild ischemia that seizure can develop in stroke patients without persisting paresis (silent infarct)[13]. Richardson and Dodge emphasized that a vascular occlusion may go unrecognized and seizure subsequently appear[14]. The patients with severe strokes, or strokes presenting with seizures, may be more likely to be admitted to hospital[1].

The distinction between early and late onset seizure has been taken into account in this study as many other investigators. Early onset seizure occurring within two weeks following stroke onset.

The study show that (25.9 %) of the seizure(s) after stroke is onset seizure (occurring with 24 hours as the onset of stroke) (Table 1), and mainly focal (Table 5). This is compatible with the result of other report were the seizure usually single, focal and readily controlled[14].

Although statistically not significant, seizures at the onset of stroke differ from early onset in that ischemic stroke is more than hemorrhagic (Table 3), focal more than generalized with no focal elements in (35.5%) of the patients (Table 5). It is well known clinically and experimentally that anything causing neuronal anoxia may precipitate seizure[14]. Onset seizure is more with ischemic stroke (Table 5) may confirm the notion that it may represent release due to focal ischemia rather than true epileptic seizure[8]. The same conclusion of Heuts et al about early and late onset seizures that, these two seizure types may differ in terms of seizures mechanism[15].

It demonstrates that a significantly lower rate of patients with early onset seizures develop another seizure i.e. epilepsy, than do patients with late onset seizures[7].This may be explained by the higher incidence of hemorrhagic stroke in early onset seizures (Table 3). Generalized seizures with no focal element are more in hemorrhagic stroke. Hemorrhagic stroke is irritative lesion rather than destructive lesions.

24.1% of patients in the study have subcortical affection (Table 1). Seizure(s) complicating basal ganglionic haemorrhage and lacunar infarction suggesting that the association between post stroke seizures and cortical damage may not be as exclusive as previously thought[1]. This may be due to the major role of basal ganglia in the control of the cerebral cortex, especially the motor area and some types of epilepsy show a close relationship between cortical and basal ganglia activity.

Although the incidence of seizures and epilepsy, are more common following cerebral infarcts or intracerebral hemorrhage, in childhood than adulthood[12]. Age group above 50 years old have significantly increased risk of having late onset seizure by five times (after controlling for the type of central nervous system insult) (Table 2) may be because of higher incidence of the stroke in advancing age. But it can be explained by the presence of brain atrophy with advancing age and a decrease in the seizure threshold which is an added factor for epilepsy after stroke. This may explain the cases of infarction with late onset seizures with no focal element

This hypothesis may be confirmed by the increase in the incidence of seizures in patient with deep lacunar infarction if there is subcortical vascular encephalopathy[5] or leukoaraisis[9]. Which is another factor can give an idea that stroke decrease the threshold to the seizure(s) rather than direct cause of epileptogenic focus.

Conclusion

1. Patients with infarction stroke in the age group above 50 years old had a significantly higher risk of suffering from seizure after stroke.

2. The onset was significantly delayed in those with cortical affection compared to those with subcortical one.
3. Age group above 50 years old significantly increases the risk of having late onset seizure.
4. Infarction as a cause of central nervous system insult, significantly increase the risk of having late onset seizure.

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PREVIOUS ABDOMINAL SURGERY AND TUBAL PREGNANCY

M.S. Al- Saadi *FRCOG*, **Ridha Turky** *D.G.O.*

Abstract

Background: Despite the technologological advances and the greater awareness leading to early diagnosis and treatment of ectopic pregnancy, still ectopic remains the primary killer of pregnant women.

Objectives To establish a correlation between previous abdominal surgery and tubal pregnancy in a sample of women with proved tubal pregnancy.

Patients & Methods: 25 patients with a confirmed diagnosis of tubal pregnancy were recruited to participate in a prospective study with a control group of 100 women of comparable demographic characteristics who had at least one full term pregnancy, regarding certain risk factors including previous abdominal surgery, history of pelvic inflammatory disease, type of contraception and previous obstetric history. Patients with previous ectopic pregnancy and those with previous tubal surgery were excluded from the study. Operative findings were recorded including site, side, tubal rupture, adhesions, associated pathology and the state of the other tube.

Results: There was a significant difference in the number of abortions being higher in the study group (36%) than the control group (14%). The number of patients with history of PID was 8(32%) in the study group & 15(15%) in the control group. Six (24%) of the study group had IUCD while 19(19%) of the control group had IUCD. We did not find a significant difference between the study group & the control group regarding to risk of tubal ectopic and previous abdominal surgery with the possible exception of appendectomy (12%) in the study group & 9% in the control group.

Conclusion: After excluding women with surgery directly on the fallopian tubes, we observed little or no excess risk associated with a history of previous abdominal surgery. The present study shows that the standard of patient's awareness, clinician's index of suspicion as well as availability of modern technologies are still poor in our community as most of the ectopics (80%) were ruptured on intervention.

Key words: Tubal pregnancy, abdominal surgery.

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Introduction

Technological advances since 1970 have helped to diagnose and treat ectopic pregnancy earlier and more consistently. Before that year ectopics ended by tubal abortion or tubal rupture that threatened the life of the patient and needed emergency surgery in most of the cases sacrificing the tube. Today 80% of ectopics are diagnosed and treated before rupture can occur[1]. Still, ectopic pregnancy remains the primary killer of pregnant women[2]. Because the number of ectopics has increased in the same 30-year period-with the fastest rise between 1970 and 1985, a fourfold growth from 17,800 to 78, 4003. It is essential that healthcare professionals be alert for ectopic pregnancy as they evaluate female patients with abdominal pain.

Some 97% of ectopics are tubal. The ampulla, isthmus and fimbria are the most common sites. Technological developments have enhanced the clinician's ability to diagnose and treat an ectopic before hemodynamic compromise can occur. First, a sensitive radioimmunoessay test is available to monitor a series of quantitative tests to screen for ectopics. These tests are sensitive even several days before a missed period. The second technical improvement in diagnosis is ultrasound, which can detect pregnancy sac implantation about 1.5 to 2 weeks following a missed period. Recent color flow doppler technology further enhances diagnostic ability. The third technical advance is the use of laparoscopy for conservative techniques such as salpingostomy, salpingotomy, segmental

Dept. Gynecology & Obstetric, College of Medicine, Al-Nahrain University.
Address correspondence to Dr. M. Al-Saadi E-mail: malka282s@yahoo.com
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excision or fimbrial expression. Further fertility is conserved, and about 60% of women will have a term pregnancy. Unfortunately the chance that an ectopic will recur is increased. Another complication that may occur is persistant trophoblastic material so monitoring HCG levels back to baseline is essential. The final technical advance has been the use of methotrexate as a medical form of therapy[3].

Aim of the study: To establish a correlation between previous abdominal surgery and tubal pregnancy in a sample of women with proved tubal pregnancy.

Patients & Methods

All women with a confirmed diagnosis of tubal pregnancy at Al-Kadhimiya Teaching Hospital during the period from 1st October 2001 to 30th June 2002 were recruited to participate in a prospective study (number 25). The total number of live birth in the department during the same period was 2617 (accounting for 9.9 tubal pregnancies per 1000 live birth). The control group consisted of 100 women of comparable demographic characteristics who had at least one full term pregnancy.

The exclusion criteria were previous ectopic pregnancy and previous abdominal surgery involving the tubes such as tubal surgery for infertility and unilateral salpingectomy.

Detailed information regarding age, education, medical and sexual history, obstetric and contraceptive history, life style, history of pelvic inflammatory disease (PID), vaginal douching, vaginal infection and any abdominal surgery was recorded.

The types of abdominal surgery assessed included appendectomy (ruptured or unruptered) Caesarean section (C/section), surgery on the ovaries and myomectomy. Other operations, such as ileostomy, exploratory laparotomy, pyloroplasty, and cholecystectomy, occurred with a relatively low frequency. These were included under "any abdominal surgery" but were not assessed separately. Observations regarding the anatomic site of the tubal pregnancy (ampullary, isthmic, fimbrial, other), laterality, complication (ruptured or not), presence of infection, endometriosis and adhesions and the state of the other tube were recorded. Data processing involved X^2 and student T test, a P value of <0.05 was regarded significant.

Results

Table 1: shows the characteristics of the study group and the control group

Characteristics		Study group		Control group	
		Number	%	Number	%
Age (Yr.)	18-25	7	28%	19	19%
	26-33	5	20%	65	65%
	=>34	13	52%	16	16%
No. of term Vaginal deliveries	1-2	7	28%	34	34%
	=>3	8	32%	50	50%
No. of abortions		9	36%	14	14%*
Contraception	IUCD	6	24%	19	19%
	C.O.C Pills	3	12%	20	20%
	Others	1	4%	5	5%
	No contraception	15	60%	56	56%
History of PID	Never	17	68%	85	85%
	Ever	8	32%	15	15%
Smoking	Former	1	4%	1	1%
	Current	-	-	-	-
Vaginal douching		2	8%	12	12%
Vaginal infection		5	20%	30	30%
*Significant P. value<0.05					

Table 2: The specific types of abdominal surgery in the two groups

Abdominal surgery	Study Group		Control Group	
	Number	%	Number	%
None	16	64%	73	73%
C/section	4	16%	16	16%
Appendectomy	3	12%	9	9%
Myomectomy	1	4%	1	1%
Ovarian surgery				
Any abdominal surgery	1	4%	1	1%
Total	25	100%	100	100%

The appendix was ruptured in the 3 patients in the study group and in 5 (55%) of the control group. Due to small number of patients with myomectomy, ovarian surgery and any abdominal surgery the findings were not included in the statistical analysis.

Table 3: The operative findings in the study group

Operative findings		No.	%
Side	Rt.	15	60%
	Lt.	10	40%
Site	Ampullary	14	56%
	Isthmic	5	20%
	Fimbrial	2	8%
	Other	4	16%
Tube	Ruptured	20	80%
	Intact	5	20%
	Hydrosalpinx (bilateral, intact)	1	4%
Adhesions		7	28%
Endometriosis		2	8%
Other tube	Normal	21	84%
	Abnormal	4	16%

Discussion

Recent testing advances and a greater awareness of risk factors for ectopic pregnancy have led to earlier diagnosis withy treatment options that can preserve fertility[4].

In the present study some of the risk factors for ectopic pregnancy were studied. We found a significant difference between the control group and the study group regarding history of previous abortion 14% and 36% respectively. Although all abortions were spontaneous miscarriage in both groups. The high figure may be explained by the occurrence of sub clinical infection following late presentation of miscarriage after several days of vaginal bleeding. The other significant finding was pelvic inflammatory disease, which was reported in 15% of the control group and in 32% of the study group.

The present study suggests that a history of abdominal surgery does not increase the risk of tubal pregnancy with the possible exception of appendectomy (12% in the study group and 9% in the control group).

Previous studies of tubal pregnancy related to a history of abdominal surgery have yielded conflicting results[5]. However, classification of types of abdominal surgery differed among these studies. One study combined all types of laparotomy whereas the others included only gynecologic operations and even tubal ligation, a procedure known to reduce the risk of tubal pregnancy[6].

After excluding women with surgery directly on the fallopian tubes, we observed little or no excess risk associated with a history of previous abdominal surgery. Our results concerning appendectomy and tubal pregnancy are in general agreement with those reported by March *et al* 1988[7], i.e., a small excess risk that could well has arisen by chance.

Regarding contraception (24%) of the study group had IUCD while 19(19%) of the control group had IUCD. The difference did not reach statistical significance. The same holds true for smoking, vaginal douching and vaginal infection.

We did not find a significant difference in the number of C/section in the study group and the control group 16% and 16% respectively. Previous C/section has been linked to a small increase in ectopic pregnancy risk[8].

In a study regarding risk factors for ectopic pregnancy in assisted reproduction. Annika Strandell 1999 reported that tubal factor infertility and previous myomectomy were the strongest independent predictors of ectopic pregnancy and that tubal factor is the stronger of the two. They suggested further study of the myomectomy issue, because the previous results have not shown this to be a consistent risk factor. They also recommend that future research examine the role of embryo placement and injection pressure of ET may play a role in the incidence of EP[9].

The present study shows that the standard of patient's awareness, clinician's index of suspicion and the availability of modern technology for diagnosis of ectopics are still poor in our community as most of the ectopics (80%) were found ruptured on intervention.

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تحفيز تمايز الخلايا الجذعية الجنينية بالهايدروكورتزون إلى خلايا شبيهة بالخلايا العصبية في الوسط الزرعي

محمود حياوي حماش[١]، انتصار نعمان وحيد[٢]

الخلاصة

خلفية الدراسة: يمكن إدامة الفعالية الكامنة للخلايا الجذعية الجنينية المشتقة من كتلة الخلايا الداخلية لأريمة الفأر في الوسط الزرعي. في الوسط الزرعي و في ظروف ملائمة تظهر الخلايا الجذعية الجنينية تمايزا إلى أنواع الخلايا المنحدرة من الطبقات الجرثومية الأولية للجنين.

هدف الدراسة: تسجيل انحدار الخلايا الشبيهة بالخلايا العصبية من الخلايا الجذعية الجنينية للفئران و التي بقيت محافظة على حالة عدم التمايز خلال عمليات الزرع الثانوية المتواصلة.

طريقة العمل: تم الحصول على خلايا جذعية جنينية للفأر من استزراع الأريمة، ثم أخضعت هذه الخلايا إلى البروتوكول الذي يسمح بتمايزها خارج الجسم للحصول على تجمعات و اجسام جنينية. بعد الزرع الثانوي لهذه التجمعات و الأجسام الجنينية و تكوينها طبقة من الخلايا، يتم معاملتها بالهايدروكورتزون و لمدة محددة.

النتائج: أدت المعاملة بالهايدروكورتزون إلى ظهور لاحق للخلايا الشبيهة بالعصبية في الوسط الزرعي.

الاستنتاج: الهايدروكورتزون، يلعب دور مهم هنا لتوجيه تمايز الخلايا الجذعية الجنينية إلى المسار العصبي.

مفتاح الكلمات: الخلايا الجذعية الجنينية للفأر، التمايز العصبي، هايدروكورتزون.

[١]قسم التشريح البشري (كلية الطب-جامعة النهرين)
[٢] مركز البحوث الطبية (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣ ، العدد ٣، ص ١٠٠-١٠٧

المعاملة بالأوكسيتوسين في الحي تحفز الظمور المستحث للتوثة في الفئران

لندا صلاح الدين فوزي[1] ،علي حيدر صاحب عبد[2]

الخلاصة
خلفية الدراسة: لقد وجد بأن الاوكسيتوسين ينتج بواسطة الخلايا الظهارية التوثية و أعتبر مستضدا شخصيا لعائلةالهورمونات النخامية العصبية في التوثة. لقد أفترض أن هذا الهرمون النخامي العصبي يمتلك دورا ثنائيا و ذلك في كل من عمليتي الاختيار الإيجابي و الاختيار السلبي للخلايا التوثية. تبذل في الوقت الحاضر محاولات لتوضيح تأثيرات المستويات العالية من الاوكسيتوسين على الوظيفة المناعية للنماذج الحيوانية.
هدف الدراسة: لدراسة تأثيرات الاوكسيتوسين على الجوانب النسجية و الفسلجية للتوثة في الجسم الحي.
طريقة العمل: تم حقن عشرين فأرا أبيضا من الذكور بعمر أربعة أسابيع بهرمون الاوكسيتوسين (بجرع مقدارها ٠,٢ مل/ يوم و بتراكيز تبلغ ٠,٥ ، ١,٠ أو ٢,٠ وحدة عالمية و لمدة ١٠ أيام). تمت الدراسة النسجية باستخدام طرقة التصبيغ بألهيماتوكسيلين– أيوسين بالإضافة إلى التصبيغ باستخدام تفاعل شف–حامض البيريوديك. أجريت القياسات المجهرية باستخدام عدسة القياسات الخلوية المدرجة.
النتائج: تأثرت الجوانب الجوانب الخلوية و التركيبية ووزن التوثة بشكل ملحوظ عند استخدام كل من التراكيز الثلاثة من هرمون الاوكسيتوسين. تسبب الهرمون بزيادات في الموت الخلوي و التنخر النسجي و الفعالية البلعمية و تكوين الأكياس في نسيج التوثة بالإضافة إلى ظهور علامات النزف الدموي في النسيج و الاحتقان داخل الشعيرات الدموية في الغدة. لوحظت كذلك حالات انخفاض في وزن التوثة و في سمك قشرة الغدة و كذلك زيادة في أقطار جسيمات هسال.
الاستنتاج: أن الأوكسيتوسين (بالجرع المستخدمة في هذه الدراسة) يكون قادرا على تحفيز الظمور المستحث للتوثة. توفر النتائج أيضا دعما في الحي لوجود دور للأوكسيتوسين في عملية الاختيار السلبي للخلايا التائية داخل التوثة.

مفتاح الكلمات: الاوكسيتوسين،التوثة،الفئران

[1]قسم البيولوجي الطبي (كلية الطب- جامعة النهرين)
[2]قسم علوم الحياة (كلية العلوم للبنات – جامعة بغداد)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣ ، العدد ٣، ص ١٠٠-١٠٧

التغيرات التركيبية لجلد الفئران تحت تأثير نقص البروتين

خالدة كاظم جبارة، نوال عبدالله، كوثر حسن

الخلاصة
خلفية الدراسة: نقص البروتين من امراض نقص التغذية الشائعة و الذي يؤثر على النمو، البقاء و وظائف الجسم الأخرى.
هدف الدراسة: هذة الدراسة خططت لمعرفة إمكانية تأثير نقص البروتين على التركيب النسيجي لطبقات الجلد المختلفة.
طريقة العمل: تم اخذ عينات من منطقة البطن من جلد (٢٠) فأر ابيض من سلالة BALB/C تتراوح أعمارهم بين (٨-١٢) أسبوع كانت هذه الفئران معرضة لتغذية فيها نقص حاد في البروتين لمدة ٤ اشهر لغرض معرفة تأثير نقص البروتين على التركيب النسيجي للجلد . و كذلك تم اخذ عينات أخرى من منطقة البطن ايضا من (٥) فئران من نفس السلالة و الجنس كانت تتغذى على غذاء طبيعي بوجود البروتين.
النتائج: بعد أجراء الطرق الروتينية للعينات من أعداد الشرائح النسجية و صبغها بصبغات مختلفة أظهرت النتائج تغيرات متعددة على تركيبة الجلد النسيجية في الفئران المعرضة إلى سوء التغذية، من هذه التغيرات تضخم في الطبقة الخارجية المتقرنة للجلد و كذلك تضخم في الطبقة الحبيبية و انتشار و تراكم صبغة الميلانين في الطبقة القاعدية و بينت النتائج أيضا فقدان كبير في شعر الجلد و تقصف في حويصلات الشعر، و تجمع كبير في الألياف الكولاجينية في منطقة البشرة. لم تظهر أي تغيرات في الغدد العرقية و الغدد الدهنية تحت مستوى المجهر الضوئي.
الأستنتاج: اظهرت النتائج ان كل عمليات تصنيع البرونين و حامض الدنا في الجلد للفئران التي تعاني من نقصان التغذية قد تضررت بفعل عدد الخلايا (موت الخلايا) و ليس بسبب اختزال حجم الخلايا.

مفتاح الكلمات: جلد الفئران، تغييرات نسيجية، نقص البروتين.

فرع التشريح و الأنسجة و الأجنة (كلية الطب-جامعة البصرة)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣، ص ١٠٠ ـ ١٠٧

دراسة بالمجهر الضوئي والمجهر الالكتروني الماسح لتكوُّن طبقة الأديم السفلي في المراحل المبكرة لجنين الدجاج

محمود حياوي حماش[1]، ميسون عبد الأمير السلمان[2]

الخلاصة:
خلفية الدراسة: يعتبر تكون الطبقات الجرثومية في المراحل المبكرة من النمو الجنيني خطوة أساسية، ولازالت آلية تكون الطبقات تخضع للدراسة في جنين الدجاج، الذي يعتبر نموذجاً لنمو الفقريات العليا، يشكل تكوُّن طبقة الأديم السفلي مشكلة من حيث مصادر الخلايا التي تساهم في تشكيلها و من حيث آلية تكوُّ نها كطبقة مستمرة.
هدف الدراسة: دراسة بملاحظة مقربة لتفاصيل تكون طبقة الأديم السفلي باستخدام المجهر الضوئي والمجهر الالكتروني الماسح.
طريقة العمل: حضنت بيوض الدجاج لمدة ٥–٢٥ ساعة بدرجة حرارة ٥ر٣٧مْ و تم فحص القرص الجنيني لتحديد المرحلة وفقاً لجدول هامبرغر و هاملتون، بعد ذلك تم تثبيت الأجنة و عولجت للفحص بالمجهر الضوئي و المجهر الالكتروني الماسح.
النتائج والاستنتاج: يبدأ النمو الابتدائي من الحافة الخلفية (الذيلية) للقرص الجنيني مكوناً تركيباً يشبه الدرع تحت الطبقة السطحية. يستمر الدرع في التوسع بالاتجاه الرأسي من المرحلة (١) إلى المرحلة (٣) حسب جدول (همبرغر و هاملتون). يُعزز هذا التوسع بخلايا منبعجة من طبقة الأديم الخارجي، و كذلك من المنطقة الحافُية. تتصل الخلايا مع بعضها تدريجياً مكونه مناطق اتصال قريبة و من ثم طبقة مستمرة.

مفتاح الكلمات: جنين الدجاج، القرص الاديمي، طبقة الأديم المتوسط.

[1]قسم التشريح البشري (كلية الطب-جامعة النهرين)
[2]قسم البايولوجي الطبي (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣، ص ١٠٠ ـ ١٠٧

دراسة التغيرات الحاصلة في النقل الايوني لكريات الدم الحمراء في الحمل المسبب لارتفاع ضغط الدم

إبتسام طاهر العبوسي

الخلاصة
خلفية الدراسة: ان مشاكل التقلص الوعائي و التخثر الحاصل في الحمل المصحوب بارتفاع ضغط الدم ركز على قياس نسب المركبات الامينية الحيوية لفهم و شرح الطرق المسببة لاستحداث ارتفاع ضغط الدم. بالاضافة الى ذلك يعتقد ان احد الاسباب التي لها دور في احداث هذا الخلل هو التوازن والنقل الايوني. انزيمات الاتباز **(ATPases)** هي مجموعة كبيرة من الانزيمات الناقلة للايونات عبر جدار الخلية، و هي مسؤوله عن التوازن الايوني داخل الخلية. تعمل هذه الانزيمات كمضخات فعالة تستهلك مركبات الطاقة العالية **(ATP)**.

هدف الدراسة: لتقييس فعالية هذه الانزيمات و التغيرات الحاصلة لها و دورها في استحداث ضغط الدم عند الحوامل.

طريقة العمل: تم اختيار ٧٦ حامل لقياس فعالية انزيمات الاتباز ثم تم تقسيم الحوامل الى مجموعتين: ٣٦ مريضة مصابة بتسمم الحمل في معدل عمري (5 ± ٥) واسابيع حمل (٣٠ ± ٤). ان معدلات ضغط الدم لهذه المجموعة كان ١٠٠/١٥٥ يعالجون بادوية خافضة للضغط. مجموعة اخرى تتكون من ٢٠ حامل في معدل عمري (٢٩ ± ٥) واسابيع حمل (٢٨ ± ٦) تعاني هذه المجموعة من ارتفاع متوسط في ضغط الدم و يعالجون بواسطه الحمية دون الادوية. اما المجموعة المسيطرة فكانت تتكون من ٢٠ حامل يتمتعن بصحة جيدة و حمل طبيعي.

النتائج: لقد تمت دراسة الانزيمات الناقلة لايوانات الصوديوم، البوتاسيوم و الكالسيوم. ان فعالية انزيمات الاتباز الناقلة للصوديوم و البوتاسيوم المقاسة بـ (مايكرومول **Pi**/ ملغم بروتين/ ساعة) وجدت منخفضة بدرجة معنوية كبيرة لفئتي الحمل الاولى و الثانية عند مقارنتهما مع المجموعة المسيطرة. اما انزيم الاتباز الناقل للكالسيوم فقد وجد انه قد ارتفع عند النساء المصابات بارتفاع متوسط في الضغط، و لكنه وجد منخفض جدا عند المصابات بتسمم الحمل.

الاستنتاج: ان قلة فعالية اتباز الصوديوم و البوتاسيوم يسبب ارتفاع الصوديوم داخل الخلية و الذي يسبب الشد الخلوي و ازدياد المقاومة للخلايا الوعائيه بالاضافة الى ذلك انخفاض فعالية انزيم الناقل للكالسيوم في الحوامل المصابات بتسمم الحمل يسبب ارتفاع الكالسيوم داخل الخلايا مسببا في ازدياد التقلصات الوعائية مسببة لارتفاع الضغط.

مفتاح الكلمات: ارتفاع ضغط الدم، تسمم الحمل، المضخات الأيونية، انزيمات الأتباز.

قسم الكيمياء والكيمياء الحياتية (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣، ص ١٠٠ ـ ١٠٧

دراسة بعض النيوكليسيدات و النيوكليسيدات المحورة فى مرضى سرطان الدم الحاد

علاء كريم محمد[١]، سرمد بهجت ديكران[٢]، كوثرعبد الرزاق الزبيدى[٢]

الخلاصة:
خلفية الدراسة: تقنية كروموتوغرافيا السائل ذات الاداء العالى تسمح بالتقدير الكمى للتراكيز الضئيلة من النيكلوسيدات و النيكلوسيدات المحورة فى مختلف السوائل الجسمية. ان قياس مستوى النيكلوسيدات مثل الاينوسين، الكوانوسين و البسيدويوردين اضافة للقاعدة الهيبوزانثين في مصل الدم كموشر للاورام اكثر فائدة من قياسها في نماذج الادرار بسبب عدم تاثرها بالعوامل المختلفة.
هدف الدراسة: تعين بعض النيكلوسيدات و القواعد بتقنية كروموتوغرافيا السائل ذات الاداء العالي و علاقة تركيزها في مصل دم الاشخاص المصابين بسرطان الدم الحاد.
طريقة العمل: تضمنت الدراسة ٤٣ شخصا مقسمين الى ٩ اشخاص مصابين بسرطان الدم من نوع (myelogenous) معدل الاعمار يتراوح بين (٩–٤٥) سنة، ١٣ شخص مصابين بسرطان الدم من نوع (lymphoplastic) معدل العمر يتراوح بين (٧–٥٨) سنة، و مقارنة النتائج مع ٢٢ شخص سليم تتراوح اعمارهم بين (٨–٦٠) سنة. تم قياس محتوى المصل بتقنية كروموتوغرافيا السائل ذات الاداء العالي.
النتائج: نتائج الدراسة تشير الى ان معدل مستوى كل من الاينوسين، الكوانوسين و الهايبوزانثين يزداد فى مصل دم المرضى المصابين بسرطان الدم عند مقارنتها مع الاشخاص الاصحاء و كذلك تشير الدراسة الى ان مستوى تركيز البسيدويوردين فى مصل الدم كناتج من عملية تهديم الحامض النووي الناقل tRNA يزداد بصورة ملحوظة عند مقارنتها مع تركيزة في مصل دم الاصحاء.
الاستنتاج: لذلك يمكن الافتراض بان مستوى هذة المركبات في مصل دم المرضى المصابين بسرطان الدم يمكن ان تكون كموشر يتنبأ به الى فعالية و مراحل بدء ظهور المرض و الى فعالية العلاج في المراحل الابتدائية مما يتطلب مزيد من الدراسة حول استخدام تلك الجزيئات كموشر للاورام.

مفتاح الكلمات: النيكليوسيدات المحورة، البسيدويوريدين، سرطان الدم.

[١] قسم الكيمياء (كلية التربية-ابن الهيثم- جامعة بغداد)
[٢] قسم العلوم الصيدلانية (كلية الصيدلة- جامعة تكريت)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣، ص ١٠٠ ـ ١٠٧

تأثير ليفاميزول على استماتة الخلايا السعتريّة

أسراء فائق السامرائي

الخلاصة
خلفية الدراسة: ليفاميزول هو عقار مضاد للديدان، أستعمل حديثا كمغير مناعي لكثير من الأمراض مثل المتلازمة الكلائية، و يستعمل كذلك كمساعد في سرطان القولون و المستقيم و لكن كيفية عمله غير معلومة. الأستماتة هي عملية فسلجية مهمة تتضمن حذف خلايا معينة و تتميز بأنقسام خاص للدنا.
هدف الدراسة: دراسة تأثير ليفاميزول على الخلايا السعترية، و الفعل المحتمل لهذا العقار في حث الأستماتة، و علاقته بفعالية أنزيم الكالسيوم أي تي باز.
طريقة العمل: تضمنت الدراسة ١٢ جرذا (٦ منها استعملت كمجموعة مقارنة و ٦ عولجت بالليفاميزول ٢ ملغم/كغم لمدّة ٦ أسابيع). تمّ قياس فعّالية أنزيم كالسيوم أي تي باز في عينات الدم و أنسجة الغدة السعتريّة. كما تمّ فحص العينات بالمجهر الضوئي و المجهر الالكتروني.
النتائج: كان هناك تغيرات الأستماتة في المجموعة التي تلقّت علاج ليفاميزول و في أكثر من ٦٠٪ من الخلايا. إن فعالية أنزيم كالسيوم أي تي باز في نسيج و دم الجرذان قبل و بعد الليفاميزول، أظهرت تغيرات معنوّية في المجموعة التي تلقّت ليفاميزول $p<0.005$.
الأستنتاج: يستطيع ليفاميزول احداث استماتة الخلايا السعترية للجرذ عن طريق تقليل فعّالية أنزيم كالسيوم أي تي باز.

مفتاح الكلمات: ليفاميزول، كالسيوم أي تي باز، الأستماتة.

قسم الفسلجة (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣ ص ١٠٠ ـ ١٠٧

دور جزيئات الوسط الكبيرة في عملية تجمع وترسب كريات الدم الحمراء

رويدة عبد الأمير[١] ، تقي الواجدي[٢] ، فاخر سلمان العاني[١]

الخلاصة

خلفية الدراسة: تجمع كريات الدم الحمراء ظاهرة فسيولوجية مهمة في الدورة الدموية. هذه الظاهرة تمثل الخصائص الاساسية للدم الطبيعي و التي تلعب دور مهم في جهاز الدوران و خاصة في الاوعية الدموية الشعرية .

هدف الدراسة: لبيان دور جزئيات الوسط الكبيرة على تجمع و ترسب كريات الدم الحمراء.

طريقة العمل: أجريت الدراسة الحالية على ٤١ شخص سليم. تتلخص طريقة العمل بمرور شعاع الليزر بعينة من خليط الدم و يتم حساب شدة أشعة الليزر النافذة بشكل مستمر خلال عملية التجمع و الترسب تحت تأثير مجال الجاذبية. و تقاس الفترة الزمنية لمرحلة تكون اللفة و تكون التجمع بمتجه او ثلاث متجهات.

النتائج: أظهرت هذه الدراسة ان الوقت اللازم لمراحل التجمع و ترسب لكريات الدم الحمراء في وسط من الدكستران، (٥٠٠,٠٠٠) وزن جزيئي، اقل من الوقت اللازم لنفس مراحل تجمع و ترسب كريات الدم الحمراء الموجودة في بلازما طبيعية. الفايبرينوجين الموجود في البلازما له دور في تكون اللفة، اما البروتين الآخر (اميونوكلوبيولن) له دور في تكون التجمع بمتجه او ثلاث متجهات.

ألأستنتاج: تم ملاحظة أن وجود الجزئيات الكبيرة و البروتينات و خصائصها (الوزن الجزيئي و الشكل) له دور كبير في كل مرحلة من عملية التجمع.

مفتاح الكلمات: تجمع كريات الدم الحمراء، معدل الترسب، جزيئات الوسط الكبيرة، أشعة الليزر.

[١]قسم الفسلجة (كلية الطب-جامعة النهرين).
[٢]قسم الفسلجة (كلية الطب-الجامعة المستنصرية).

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣ ص ١٠٠ ـ ١٠٧

استخدام الكروم اكار اورينتيشن في الكشف عن البكتيريا في نماذج الادرار

منال عدنان حبيب[١]، الهام القيسي[٢]، ليلى شعيب العمر[١]

الخلاصة

خلفية الدراسة: يعتبر **CHROM agar Orientation** من الاوساط التي تستخدم للكشف ولتمييز النمو البكتيري في النماذج السريرية.

هدف الدراسة: تقييم **CHROM agar Orientation** في تشخيص البكتيريا في نماذج الادرار مقارنة بالاوساط التقليدية المستخدمة لهذا الغرض.

طريقة العمل: استخدمت ثلاثة اوساط**MacConkey agar** & **Blood agar, CHROM agar Orientation** للزرع المباشر ل ٤١٥ نموذج ادرار من حالات التهاب المجاري البولية.

النتائج: نجح **CHROM agar Orientation** في الكشف عن البكتيريا في جميع نماذج الادرار والتي تم الكشف عنها بالاوساط المرجعية ولكن للتمييز بين اصناف البكتيريا نحتاج الا اختبارات كيميائية حياتية اضافية.

الاستنتاج: ان وسط **CHROM agar Orientation** هو وسط ممتاز للكشف عن البكتيريا في نماذج الادرار كزرع نقي او مختلط ولذلك يمكن استخدامها بدلا من الاوساط التقليدية التي تستخدم روتينيا في تشخيص الاخماج المسببة لالتهاب المجاري البولية.

مفتاح الكلمات: كروم اكار اورينتيشن، ادرار.

[١]قسم الاحياء المجهرية الطبية (كلية الطب- جامعة النهرين)
[٢]قسم الصيدلة و التقانة الطبية (جامعة البترا-عمان-الأردن)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣، ص ١٠٠ ـ ١٠٧

مقارنة بين نوعين من مستضدات المبيضات الفطرية المستخدمة في تشخيص مرض المبيضات الجهازي

أزهار عبد الفتاح الأطرقجي

الخلاصة
خلفية الدراسة: ان اكثر الخصائص الطبية للأمراض الفطرية غير كافية لأعطاء تشخيص كامل للمرض، لذا فالمعلومات المتوفرة يجب ان تكون كافية لعلاج المرض الجهازي. ان مرض المبيضات الجهازي يصيب الأشخاص ذوي المناعة الضعيفة و هناك طرق عديدة تستخدم كتشخيص مصلي و لكن معضمها مخيب للآمال. ان احد اهم المستضدات التي يمكن الأعتماد عليها في تشخيص مثل هذه الحالات هو المستضد الهيولي **(cytoplasmic)** .
هدف الدراسة: المقارنة بين نوعين من المستضدات المعزولة من المبيضات الفطرية و استخدامها في التشخيص المصلي لمرض المبيضات الجهازي.
طريقة العمل: تم استخدام عزلة من المبيضات الفطرية و هي العزلة **(ATCC 10231)** و المزروعة على وسط **Sabouraud borth** . تم جمع عينات الدم ل ١٠٠ مريض بمرض ابيضاض الدم الحاد بنوعيه الليمفاوي و النخاعي لمرضى راقدين في مختلف المستشفيات في العراق. تم جمع قشع او مسحة فمية من هؤلاء المرضى. تم زرع عينات الدم في وسط **(Brain heart infusion)** اما مادة **Phosphate buffer saline** فكانت بدارىء ٧ز٠ و مادة الأكاروز **agarose** فكانت بتركيز ١ز٥٪.
النتائج: ثمانية مرضى من ١٠٠ كانوا مصابين بمرض الفطريات الجهازي و قد تم تشخيصهم بأستخدام المستضد الهيولي بطريقة الترسيب، بينما اعطى المستضد المستخلص من جدار الفطر نتيجة ايجابية ل ٧٧٪ من المرضى المصابين.
الأستنتاج: المستضد الهيولي هو المستضد الملائم كتشخيص سيرولوجي لمرضى المبيضات الفطرية الجهازي.

مفتاح الكلمات: المبيضات الفطرية، مرض المبيضات الجهازي.

قسم الأحياء المجهرية الطبية (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣ ، ص ١٠٠ ـ ١٠٧

إعتماد فحص نسيج نخاع العظم في تقييم سير المرض عند مرضى لوكيميا نقّي الدم المزمن

صبح سالم المدلل[١]، سعد شوقي منصور[١]، فاطمة الراوي[٢]

الخلاصة

خلفية الدراسة: ان مرض لوكيميا نقّي الدم المزمن ناتج عن تحول خبيث في الخلايا الجذعية الموجودة في نخاع العظم. لقد تم استنباط عدة تصنيفات لغرض التكهن بشدة للمرض و التنبؤ بخط سير المرض ومن هذه التصنيفات المستعملة هي ما يسمى **Sokal Scoring System** حيث يقسم المرض اعتماداً على نسبة الاضرار **(Hazard Ratio)** الى مجموعة قليلة و متوسطة و عالية الخطورة. هذا بالاضافة الى اعتماد عدة متغيرات التي تشمل التغيرات الحاصلة في الحالة الصحية للمرض و كذلك فحص صورة الدم العامة و تقييم المتغيرات في نسيج نخاع العظم و التي يمكن بمجملها أن تؤثر على سير المرض.

هدف الدراسة: تقييم أهمية فحص عدد الخلايا المولدة للاقراص الدموية و نسبة زيادة الاليـاف في خزعة نخاع العظم على طرق سير المرض و التأكد من تأثير التغييرات النسيجية الحاصلة في نخاع العظم على نظام **Sokal Scoring** المستخدم في تقييم درجة خبث المرض و سرعة تطوره.

طريقة العمل: تم اجراء هذه الدراسة على ٦٠ مريض مصابين بمرض لوكيميا نقيّ الدم المزمن في بداية تشخيصهم و قبيل تحولهم الى لوكيميا نقيّ الدم الحاد. حيث تم دراسة الحالة الصحية للمرضى و فحص صورة الدم العامة باستخدام جهاز فحص الدم الذاتي (**Multichannel Analyzer Coulter S Plus**) و كذلك فحص خزعة من نخاع العظم لهؤلاء المرضى بعد ان تم صبغ النماذج بمادة **Hematoxylin and Eosin**لفحص خلايا نخاع العظم و بمادة **Gomori silver stain** لفحص الالياف الموجودة في نخاع العظم.

النتائج: لقد تم دراسة عدة متغيرات في خزعة نخاع العظم و قد وجد ان معدل النسيج الذي يحتوي على خلايا المولدة الدم هو ١٧,٦٨ ± ٧٨,٩٦ و معدل عدد الخلايا المولدة لاقراص الدم (**megakaryocyte**) هو ٤٠,٨±٨٥,٢ / ملم^{٢} و طبقا لمعادلة **Sokal scoring** قسّم المرضى الى ثلاثة مجاميع و هي المجموعة القليلة الخطورة (١٣ حالة) و مجموعة متوسطة الخطورة (٣٠ حالة) و اخرى شديدة الخطورة (١٩ حالة). و كان هناك فرق واضح بين هذه المجاميع فقد لوحظ ان المجموعة التي تشمل المرضى ذو الخطورة العالية الخطورة يعانون من سرعة انتشار المرضى و تدهور حالتهم الصحية السريع.

الاستنتاج: هذه الدراسة ثبتت اهمية فحص خزعة نخاع العظم و بالاخص فحص معدل عدد الخلايا المولدة لاقراص الدم و نسبة زيادة الاليـاف الموجودة في نخاع العظم و التي كانت على علاقة مباشرة بالحالة الصحية للمرضى في بداية المرض و ساعدت في اضافة متغيرات جديدة لنظام **Sokal scoring** الذي يستخدم في تصنيف المرض حسب درجة خبث المرض و سرعة تطوره.

مفتاح الكلمات: لوكيميا نقّي الدم المزمن، خزعة نخاع العظم، تقييم مسار المرض، درجة خطورة المجاميع.

[١]قسم الباثولوجي (كلية الطب-جامعة النهرين)
[٢]مستشفى الكرامة التعليمي

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تاثير مجموع حجم الكريات الحمر على وقت فحص النزف الجلدي و على الصفيحات الدموية عند المرضى المصابين بفقر الدم

صبح سالم المدلل[١]، سعد شوقي منصور[١]، فتوة عزيز[٢]

الخلاصة
خلفية الدراسة: ان قياس النزف الجلدي هو فحص شائع لدراسة تفاعل الصفيحات الدموية مع الأوعية الدموية في عملية وقف النزف، كما ان الكريات الحمر لها تاثير مهم في تفاعل الأقراص الدموية مع الأوعية الممزقة او الأوعية ذات الجدران الغير الملساء. و قد وجد ان عملية نقل الدم للمريض تساهم في اصلاح عمل الصفائح الدموية و التقليل من وقت فحص النزف الجلدي.
هدف الدراسة: دراسة تاثير مجموع حجم الكريات الحمر على النزف الجلدي في الأفراد الطبيعيين و المصابين بفقر الدم الذين ليس لديهم اختلال في وظائف الكلية و عدد اقراصهم الدموية اكثر من ١٠٠X ١٠ ٩ / لتر. و كذلك تم دراسة العلاقة بين عدد الصفائح الدموية و مجموع حجم الكريات الحمراء.
طريقة العمل: تم دراسة ٧٢ شخصا، بينهم ٢٣ شخصا سليما و ٢٩ شخصا مصابا بفقر الدم، و جميعهم ليس لديهم اختلال في وظائف الكلية و عدد صفائحهم اكثر من ١٠٠X ١٠ ٩ / لتر. تم فحص وقت النزف الجلدي بطريقة **Ivy's Method** باستعمال جهاز صغير مزود بنابض و شفرة مخصص لقيلس وقت النزف الجلدي. تم قياس مجموع حجم الكريات الحمر و عدد الصفائح الدموية بالطرق المعتمدة في المختبرات. بالنسبة لقياس نسبة اليوريا في الدم فقد تم استعمال طريقة **Nissler** و الكرياتنين في الدم ب **Sodium Tungistate**.
النتائج: وجدت علاقة عكسية مهمة بين مجموع حجم الكريات الحمر و بين فحص وقت النزف الجلدي في المرضى المصابين بفقر الدم و عدد اقراصهم اكثر من ١٠٠X ١٠ ٩ / لتر و ليس لديهم اختلال في وظائف الكلية. و جدت ايضل علاقة عكسية بين مجموع حجوم الكريات الحمر و بين الأقراص الدموية و كانت هذه العلاقة اكثر اهمية احصائيا في المرضى المصابين بفقر الدم.
الأستنتاج: تعكس نتائج البحث المتغيرات الحاصلة في الأوعية الدموية و الدم و التي ممكن ان تساعد في وقت النزف او تؤدي الى التجلط في الأنسان.

مفتاح الكلمات: مجموع حجم الكريات الحمر، عدد الصفيحات الدموية، وقت فحص النزف الجلدي.

[١] قسم علم الأمراض (كلية الطب-جامعة النهرين)
[٢] كلية الصيدلة-الجامعة المستنصرية

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الملخص ات العربية ة
مقالات مبتكرة

السرطان والعادات الغذائية

آمال سويدان[1]، احمد عباس العزاوي[2]، عبد الحسين مهدي الهادي[1]

الخلاصة
خلفية الدراسة:يعتبر السرطان المسبب الثاني للوفيات بعد أمراض القلب والشرايين. الكثير من انواع السرطان (٨٠٪) لها علاقة بنوعية غذاء الانسان.
هدف الدراسة: التحري و الكشف عن العادات الغذائية الخاطئة لعينة من المرضى المصابين بالسرطان. دراسة الحالة التغذوية و خصائص ديموغرافية أخرى و العلاج بالفيتامينات و المعادن لغرض الوصول إلى أفضل حالة صحية لمثل هؤلاء المرضى.
طريقة العمل: تم اختيار عينة من المرضى البالغين المصابين بأنواع مختلفة من السرطان في عيادة الأورام في مستشفى الكاظمية التعليمي. و تم الاستفسار عن الخصائص الديموغرافية و العادات الغذائية اضافة الى فحص الوزن و الطول.
النتائج: عدد الذين يتناولون الأسماك ثلاث مرات في الأسبوع ٢ (٣٪) و مرتين في الأسبوع ٦ (١٠٪) و مرة واحدة في الأسبوع ١٠ (١٦٪) و مرة في الشهر ٢٢ (٣٥,٥٪) و نادرا ٢٢ (٣٥,٥٪) و لا يوجد من يتناول الأسماك يوميا. عدد الذين يتناولون الفواكه يوميا ٢٣ (٣٧,١٪). عدد الذين يتناولون الخضراوات يوميا ٣٢ (٥١,٦٪). عدد الذين يتناولون زيت الزيتون نادرا ٤٤ (٧١٪).
الاستنتاج: وجود العديد من العادات الغذائية الخاطئة مثل قلة تناول كل من الأسماك و زيت الزيتون و الفواكه و الخضراوات. و لكل من هذه المواد دور معروف في الحد من النمو السرطاني و خفض نسبة الإصابة بالمرض.

مفاتيح الكلمات: العادات الغذائية، التغذية، تقييم الحالة التغذوية، السرطان.

[1]قسم طب المجتمع (كلية الطب-جامعة النهرين)
[2]قسم الطب الباطني(كلية الطب-جامعة النهرين)

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الملخصـ ات العربيـ ة
مقالات مبتكرة

دراسة طبية عدلية لتسمم جماعي بالهايوسين

طريف سرحان الغريري[1]، فائق امين بكر[2]، معتز عبد المجيد القزاز[1]

المجلة العراقية للعلوم الطبية

الخلاصة
خلفية الدراسة: يعزى التسمم الغذائي الى العديد من الأسباب أكثرها شيوعا هو التلوث البكتيري و الذي يبدأ بالتهاب حاد في الجهاز الهضمي بعد ٤٨ ساعة من تناول الطعام أو الشراب و هنالك أسباب أخرى كتحسس الجهاز الهضمي أو تناول الفواكه غيرالطازجه و الفطر او تلوث الغذاء بالمواد الكيميائية، أما التسمم بمادة الهايوسين فهو نادر الحدوث .
هدف الدراسة: تهدف الدراسة الى الكشف عن نوع المادة السمية المتناولة و بيان تأثيراتها السمية والمرضية.
طريقة العمل: أجريت الدراسة على ١٥ شخصا مصابين بأعراض تسمم حاد بعد تناولهم وجبة غذائية شعبية تدعى دوله. توفي ثلاثة منهم بعد فترات مختلفة، تم اخضاعهم بعدها للفحص التشريحي و النسيجي المرضي بالاضافة الى اجراء التحليلات السمية باستعمال الاستشراب الورقي و جهاز الاستشراب الغازي السائل.
النتائج: من خلال متابعة ١٥ شخصا أصيبوا بتسمم غذائي حاد نتيجة طهي الطعام مع مادة نباتية سامة بطريقة الخطأ توفي ٣ منهم بعد فترات متفاوتة كانت أعمارهم ١٠ أشهر، ١سنة و ٣ سنوات بينما اكتسب المتبقي منهم الشفاء التام بعد فترات تراوحت بين ٦ الى ٢٤ ساعة. أظهر التشريح الطبي العدلي على المتوفين منهم وجود نزوف دموية دقيقة في المعدة و الدماغ و القلب و الرئتين كما أظهرت فحوصات المقاطع النسيجية مجهريا وجود احتقان و نزوف موضعية دقيقة من الدماغ و الرئتين مع تغييرات شحمية و تنخر واسع في الكبد .
الاستنتاج: أظهرت الدراسة ان تأثير المادة السامة على الجسم يعتمد بصورة مباشرة على جرعة المادة السامة و عمر الضحية كما و استنتج من الدراسة ان المادة السامة تؤدي الى حدوث حالة اللاوكسية في الخلايا مع تلف كيميائي في جدران الاوعية الشعرية مسببة نزوفا دموية دقيقة و نخر في الخلايا و تأثير على انتاج الطاقة حتى في الجرعات الواطئة.

مفتاح الكلمات: التسمم بالهايوسين، السكوبولامين، اتروبابلدونا، داتورسترامونيوم

[١]قسم الطب العدلي-كلية الطب-جامعة النهرين،
[٢]معهد الطب العدلي-وزارة الصحة

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الملخص ات العربي ة
مقالات مبتكرة

داء الكيسات العدرية الصلبة في فحص الامواج فوق الصائتة: علامة جديدة بالتخطيط فوق الصوتي

نزار طه مكي[١]، عادل حمودي القيسي[٢]، يعرب ادريس خطاب[٣]

المجلة العراقية للعلوم الطبية

الخلاصة
هدف الدراسة: لتقييم اهمية علامة خط القطار كعلامة تشخيصية و تفريقية للنوع الصلب من داء الكيسات العدرية عن اورام الكبد الصلبة الاخرى.
طريقة العمل: لقد تم تقييم نتائج فحص الامواج فوق الصائتة ل ٣٣١ ورم كبدي صلب في ١٧٩ مريض. لقد تم تحليل علامة خط القطار التي اقترحت كعلامة تشخيصية لداء الكيسات العدرية الصلبة تحليلا توقعيا و قورنت مع النتائج بعد العمليات الجراحية او دراسات الرشفة المرضية.
النتائج: لقد لوحظت علامة خط القطار في ١٨ حالة كيسات عدرية صلبة تم اثباتها مرضيا من مجموع ٣٣١ كتلة كبدية صلبة و لم تلاحظ هذه العلامة في اي كتلة صلبة اخرى.
الاستنتاج: ان هذه العلامة التشخيصية في الامواج فوق الصائتة يجب ان تحثنا لتفريق داء الكيسات العدرية الصلبة عن الكتل الصلبة الاولية او الثانوية الاخرى.

مفتاح الكلمات: علامة خط القطار، داء الكيسات العدرية الكبدية الصلبة، فحص الامواج فوق الصائتة

[١]قسم الجراحة (كلية الطب-جامعة النهرين)
[٢]فرع الأشعة (كلية الطب-جامعة النهرين)
[٣]قسم علم الأمراض (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣ ، العدد ٣ ، ص ١٠٠ ـ ١٠٧

أثر عقار محفز البلازمينوجين النسيجي في علاج احتشاء العضلة القلبية

خليل بكري الكبيسي، مؤيد بشير الركابي

الخلاصة

المجلة العراقية للعلوم الطبية

خلفية الدراسة: يحدث احتشاء العضلة القلبية عادة نتيجة لانسداد حاد في احد الشرايين التاجية بسبب تخثر الدم في منطقة متضيقة بالتصلب العصيدي. و احدثت الأدوية الوريدية المحللة للخثرين مثل عقار محفز البلازمينوجين النسيجي (t-PA) ثورة في علاج هذا المرض حيث انها تساعد في فتح الشريان و تحافظ على كفاءة البطين الأيسر و تزيد من فرصة البقاء.

هدف الدراسة: لدراسة اثر هذا العقار في علاج مرضى احتشاء العضلة القلبية و مدى استجابة المرضى له و تاثير تلك الأستجابة في منع حدوث مضاعفات المرض و تأثير عوامل الخطورة لأمراض القلب في مدى الأستجابة.

طريقة العمل: تناولت الدراسة ٥٠ مريضا مصابا باحتشاء العضلة القلبية نوع (Q-wave). اعطي لهم جميعا عقار (t-PA) خلال ٦ ساعات من بداية الأعراض المصاحبة للمرض و تمت مراقبة الأستجابة من خلال ملاحظة الهبوط الحاصل في شدفة ST لأمثر من ٥٠٪ بعد ٩٠ دقيقة من بدء استعمال العلاج. تم دراسة تاثير جنس و عمر المريض و عوامل الخطورة (ارتفاع ضغط الدم، داء السكري، التدخين، و وجود تصلب شرايين سابق) على نسبة الأستجابة كما تم متابعة حدوث ذبحة صدرية بعد الأحتشاء، توسع الأحتشاء، الوفاة و مضاعفات العلاج.

النتائج: كانت نسبة الأستجابة ٣٢٪ كما لوحظ ان تقدم العمر، الجنس الأنثوي، داء السكري و ارتفاع ضغط الدم من العوامل التي تقلل نسبة الأستجابة بينما لم يكن للتدخين تاثير على نسبة الأستجابة للعلاج. ان المرضى الذين ابدو استجابة للعلاج كانت نسبة الوفيلت بينهم اقل من غير المستجيبين و كذلك الآم الذبحة الصدرية بعد الأحتشاء في حين لم يكن للاستجابة تاثير على توسع الأحتشاء، كما كانت الآثار الجانبية قليلة نسبيا بوجود حالة نزف واحدة داخل الدماغ و حالة نزف اخرى من المعدة مع ١٢٪ نزف بسيط في مناطق السحب الوريدي.

الأستنتاج: تظهر هذه الدراسة اهمية عامل الوقت لبدء العلاج في تحديد نسبة الأستجابة. كانت الفئات العمرية الشابة هي الأكثر استفادة. يقلل العقار من الذبحة الصدرية و نسبة الوفيات في الوقت الحاضر.

مفتاح الكلمات: عقار محفز البلازمينوجين النسيجي، احتشاء العضلة القلبية

قسم الطب الباطني (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣ ، ص ١٠٠ ـ ١٠٧

تعليم مرضى القلب كيفية تدبير ادويتهم

نبيل عبد الأحد انطوان[١]، عمار طالب الحمدي[٢]، حكيمة شاكر حسن[٣]

الخلاصة

هدف الدراسة: مساعدة مرضى القلب الذين بحاجة الى معرفة معلومات كافية حول ادوية امراض القلب التي وصفت لهم في المستشفى و تحقيق العناية الذاتية اللازمة لأدويتهم بعد خروجهم من المستشفى.

طريقة العمل: باستعمال نظام الأختبار القبلي و البعدي، تم فحص ٦٠ مريضا بالقلب.

النتائج: بينت النتائج زيادة في عمق المعلومات و فهم المرضى لأسماء و عمل الأدوية و متى يحتاج المريض الى طبيبه.

الأستنتاج: ان برنامج التعليم لمرضى القلب اثر في زيادة معلومات المرضى حول ادوية امراض القلب في المجموعة المدروسة. تبعا للنتائج، يوصي الباحثون التوسع في المنهاج ليشمل الأمراض و الأدوية الأخرى.

مفتاح الكلمات: تأهيل القلب، تعليم المريض، ادوية القلب.

[١] قسم الفسلجة (كلية الطب-جامعة النهرين)
[٢] قسم الطب الباطني (كلية الطب-جامعة النهرين)
[٣] قسم التمريض الباطني-الجراحي (كلية التمريض-جامعة بغداد)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣، العدد ٣، ص ١٠٠ ـ ١٠٧

مفعول البخاخ الأنفي لهرمون كالستونين في منع كسور العظام لدى النساء بعد سن اليأس

عدنان عنوز

الخلاصة
خلفية الدراسة: هشاشة العظام حالة مرضية تؤدي الى كسور و تشوهات و آلام و عوق. المرضى الذين يعانون من قلة الكالسيوم في العظام اكثر عرضة لهذه الكسور و هم بحاجة ملحة و سريعة لمنع المضاعفات.
هدف الدراسة: التحقق من فاعلية البخاخ الأنفي لهرمون كالستونين في منع حصول الكسور لدى المريضات اللاتي يعانين من هشاشة العظام بعد سن اليأس.
طريقة العمل: ثلاثمائة و عشرون مريضة تتراوح اعمارهن بين ٥٢–٧١ سنة تم اختيارهن بطريقة عشوائية و اجريت لهن فحوصات شعاعية لكل من العمود الفقري، الوركين و المعصمين لغرض التحقق من خلوها من كسور حديثة. اعطيت جرعة يومية قدرها ١٠٠ وحدة قياسية لمائة و ستون مريضة من خلال بخ الأنف بالاضافة الى غرام واحد من مادة كربونات الكالسيوم و بصورة دورية، في حين اعطيت المجموعة الثانية (١٦٠ مريضة) غرام واحد من كربونات الكالسيوم فقط. تم متابعة المجموعتين لمدة سنتين.
النتائج: اثنتان و عشرون (٧ز١٣٪) مريضة من المجموعة التي لم تتناول الهرمون اصبن بكسور في الفقرات، عنق عظم الفخذ و المعصم في حين ان مريضة واحدة فقط (٦ز٠٪) من اللاتي تناولن الهرمون قد اصيبت بكسر انكباسي في الفقرة.
الأستنتاج: ان هشاشة العظام قد تؤدي الى حدوث كسور في النساء بعد سن اليأس وان استعمال هرمون الكالستونين من نوع بخاخ الأنف هو اسلم طريق للعلاج.

مفتاح الكلمات: بخاخ كالستونين، كسور العظام، النساء، سن اليأس.

قسم الطب الباطني (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد٣ ، العدد ٣ ، ص ١٠٠ ــ ١٠٧

النوبات الصرعية عقابيل طارئة الوعائية الدماغية

عبد المطلب عبد الكريم، حسن عزيز الحمداني، عقيل جبار البهادلي

الخلاصة
خلفية الدراسة: الطارئة الوعائية الدماغية سبب هام لمرض الصرع، خاصة في كبار السن. تشير العديد من الحقائق الى ارتباط بين مرض الصرع و الأمراض الشريانية. الطارئة الوعائية الدماغية كسبب للنوبات الصرعية اعلى كثيرا في الدراسات المنفذة في العالم المتطور.
هدف الدراسة: تقييم مرضى النوبات الصرعية عقابيل طارئة الوعائية الدماغية من حيث العلاقة بين انواع النوبات الصرعية و طبيعة الأمراض الوعائية المسببة لها.
طريقة العمل: للفترة من مارس/آذار ٢٠٠٠ الى مارس/آذار ٢٠٠٢ سجل ٥٨ [٢٠ انثى (٣٤,٥٪) و ٣٨ ذكر (٦٥,٥٪)] مريض مصاب بمرض الصرع عقابيل طارئة الوعائية الدماغية في قسم الجملة العصبية لمستشفى الكاظمية التعليمي. التشخيص السريري يشمل طبيعة المرض (تجلطي او نزفي)، شمول قشرة الدماغ بالضرر او لا، اضافة الى الجهة التي اصيبت (اليسار او اليمين) بين هؤلاء المرضى، نوبات الصرع المتحققة، مقيمة و مصنفة. شمل البحث مرضى لكل الأعمار متضمنا ١٨ (٣١,١٪) تحت ٥٠ سنة و ٤٠ (٦٨,٩٪) فوق ٥٠ سنة ما مجموعه ٤١ (٧٠,٧٪) مريض احتشاء و ١٧ (٢٩,٣٪) نزف. اجري تحليل احصائي باستعمال الطرد الأحصائي للعلوم الأجتماعية اصدار ١٩٧٥.
النتائج: تضمنت النتائج مرضى النوبات الصرعية عقابيل طارئة الوعائية الدماغية مع عدم وجود نوبات سابقة للمرض الوعائي. كانت بداية المرض فوق ٥٠ سنة اكثر دلالة. شمول قشرة الدماغ بالضرر مع الأحتشاء (٩٥,١٪) اعلى كثيرا من الذي مع النزف (٢٩,٤٪). بداية المرض مع شمول قشرة الدماغ بالضرر متاخرة نسبيا. مرضى الأحتشاء في العمر اعلى من ٥٠ سنة كان معرض اكثر لحصول النوبات. الجنس و الجهة لا يحملان اي اهمية احصائية.
الأستنتاج: مرضى الأحتشاء لعمر اعلى من ٥٠ سنة كانوا معرضين لحصول النوبات الصرعية عقابيل طارئة الوعائية الدماغية و نفس هذه المجموعة معرضة اكثر لحصول النوبات المتاخرة (بعد اسبوعين). بداية حدوث النوبات متاخر في مجموعة المرضى المتضمن قشرة الدماغ بالضرر.

مفتاح الكلمات: النوبات الصرعية، عقابيل طارئة الوعائية الدماغية

قسم الطب الباطني (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣ ، العدد ٣ ، ص ١٠٠ ـ ١٠٧

عمليات فتح البطن السابقة و علاقتها بالحمل في قناة فالوب

ملكة السعدي، رضا تركي

الخلاصة
خلفية الدراسة: بالرغم من التطورات الحديثة و علاج الحمل خارج الرحم، لا يزال هذا النوع من الحمل سببا رئيسيا في وفيات النساء الحوامل.
هدف الدراسة: ايجاد علاقة بين عمليات فتح البطن السابقة و الحمل في قناة فالوب في مجموعة من النساء الحوامل.
طريقة العمل: تم دراسة ٢٥ مريضة ثبت لديهن وجود حمل خارج الرحم (في قناة فالوب) و قورنت النتائج مع ١٠٠ امراة بمواصفات ديموغرافية متشابهة ممن كان لديهن ولادة كاملة واحدة على الأقل، من ناحية عوامل الخطورة للأصابة بالحمل في الأنابيب مثل وجود عمليات فتح بطن سابقة، التهاب الحوض، انواع موانع الحمل المستعملة و تأريخ الحمل و الولادات السابقة. أستثنيت من الدراسة النساء اللواتي كان لديهن حمل خارج الرحم سابقا او سبق و ان اجريت لهن عملية جراحية للأنابيب الرحمية. تم تثبيت مكان الحمل خارج الرحم في الجهة اليمنى او اليسرى، كما تم التحري عن كون الأنبوب متمزق و هل هنالك التصاقات او حالات مرضية مرئية في الأنبوب.
النتائج: وجد فرق احصائي مؤثر بين المجموعة المدروسة بالنسبة لعدد الأسقاطات السابقة و بين المجموعة المقارنة ٣٦٪ و ١٤٪ على التوالي و كذلك بالنسبة لألتهابات الحوض حيث كان العدد ٨ (٣٢٪) في المجموعة المدروسة و ١٥ (١٥٪) في المجموعة المقارنة. بالنسبة لموانع الحمل، ظهر ان ٦ (٢٤٪) من المجموعة المدروسة كن يستعملن اللولب الرحمي مقابل ١٩ (١٩٪) من المجموعة المقارنة. اما بالنسبة لعمليات فتح البطن السابقة فلم نجد فرق احصائي مؤثر و لكن وجد فقط في حالات الزائدة الدودية فكانت النسبة ١٢٪ في المجموعة المدروسة و ٥٩٪ في المجموعة المقارنة و لكن هذه النسبة لم تكن مؤثرة احصائيا.
الأستنتاج: بعد استبعاد المريضات اللواتي لديهن عمليات سابقة على الأنبوب الرحمي، لم نجد فرق احصائي يدل على وجود خطورة للأصابة بالحمل خارج الرحم للمريضات اللواتي لديهن عمليات فتح بطن سابقة. تبين من هذه الدراسة ان وعي المريضات و الأطباء لأكتشاف الحالة مبكرا لا يزال دون المستوى و كذلك بالنسبة لوجود التقنيات الحديثة مما جعل نسبة كبيرة (٨٠٪) من الحالات تشخص بعد حدوث انفجار الحمل.

مفتاح الكلمات: الحمل خارج الرحم، قناة فالوب، عمليات فتح البطن.

قسم النسائية و التوليد (كلية الطب-جامعة النهرين)

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣ ، العدد ٣، ص ١٠٠ ـ ١٠٧

المجلة العراقية للعلوم الطبية
قائمة المحتويات

مقالات مبتكرة

المجلة العراقية للعلوم الطبية ٢٠٠٤ م، المجلد ٣ ، العدد ٣،
ص

المجلد الثالث، العدد الثالث، ١٤٢٤ هـ، ٢٠٠٤م

المجلة العراقية للعلوم الطبية

رئيس هيئة التحرير

طارق إبراهيم الجبوري

هيئة التحرير الأستشارية

أنعم رشـــيد الصالحـــي
حكمت عبد الرســـول حاتم
طارق إبراهيم الجبـــوري
عبد الحسين مهدي الهادي
عبد المطلب عبد الكريـــم
علاء غني حســـــــين
غسان الشـــــــماع
فائق حسين محمـــــد
فاروق حســـــن الجواد
ملكة سلمان الســـعدي
نشأت عزيز نشـــــــأت

هيئة التحرير

خالد عبد الله رئيس التحرير
اكثم رشيد الصالحـــــي محرر
شذى حســـــين علي محررة
خالد طارق حمدي النائـــب محـــــــــــر
فرقد بدر حمدان محـــــــــــرر

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رقم الإيداع في دار الكتب و الوثائق ببغداد ٧٠٩ لسنة ٢٠٠٠

الهيئة الأستشارية

اسامة نهاد رفعت (الهيئة العراقية للأختصاصات الطبية)

أكرم جرجيس (جامعة الموصل)

ألهام الطائي (الجامعة المستنصرية)

امجد داود نيازي (الهيئة العراقية للأختصاصات الطبية)

أميرة شبر (الجامعة المستنصرية)

ثامر أحمد حمدان (جامعة البصرة)

حسن أحمد حسن (جامعة النهرين)

حكمت الشعرباف (جامعة بغداد)

خالد عبدالله (جامعة النهرين)

داود الثامري (جامعة النهرين)

راجي الحديثي (الهيئة العراقية للأختصاصات الطبية)

رافع الراوي (جامعة النهرين)

رجاء مصطفى (الجامعة المستنصرية)

رياض العزاوي (الجامعة المستنصرية)

زكريا الحبال (جامعة الموصل)

سركيس كريكور ستراك (جامعة البصرة)

سرمد الفهد (جامعة بغداد)

سرمد خوندة (جامعة بغداد))

سميرة عبد الحسين (جامعة تكريت)

طاهر الدباغ (جامعة الموصل)

ظافر زهدي الياســـين (جامعة بغداد)

عبد الا له الجوادي (جامعة الموصل)

علي فخري الزبيدي (جامعة النهرين)

فوزان النائب (الجامعة المستنصرية)

محمود حياوي حماش (جامعة النهرين)

نجم الدين الروزنامجي (الهيئة العراقية للأختصاصات الطبية)

نزار طه مكي (جامعة النهرين)

نزار الحسني (الهيئة العراقية للأختصاصات الطبية)