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Iraqi Journal of Medical Sciences

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Issued Quarterly

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Prevalence of Celiac Disease in Developing Countries

Samar A. Al-Shami *MBChB, MSc*

Dept. of Pathology and Forensic Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Celiac disease (CD) is an autoimmune disorder, which affects genetically predisposed individuals upon the ingestion of gluten. So, it is the result of both environmental (gluten) and genetic factors (carriers of HLA-DQ2 and DQ8 haplotypes). A duodenal biopsy with positive serology is the gold standard for the diagnosis of CD. Celiac disease world geographical distribution seems to have followed the spread of wheat consumption in addition to the migratory flows of mankind. Following the application of simple serological tests for the diagnosis of CD in the 1980s, it gradually became clear that the prevalence of CD in different countries in the Middle East, North Africa and India is almost the same as that in Western countries. A high index of suspicion for CD should be maintained in all developing countries for patients who present with chronic diarrhea or iron deficiency anemia. The prevalence of CD varies with sex, age, and geographic location. The global prevalence of CD has increased over time from 0.6% in 1991 to 2000 to 0.8% between 2001 and 2016. According to that, there is a need for population-based prevalence studies in many developing countries especially middle east to estimate the burden of CD properly.

Keywords Celiac disease, gluten, HLA, prevalence, meta-analysis, Middle East, developing countries.

Citation Al-Shami SA. Prevalence of celiac disease in developing countries. Iraqi JMS. 2017; Vol. 15(4): 324-326. doi: 10.22578/IJMS.15.4.1

List of abbreviation: CD = Celiac disease, HLA-DQ2 and DQ8 = Human leukocyte antigen serotype group/ cell surface receptor proteins

Celiac disease (CD) is an autoimmune disorder which affects genetically predisposed individuals upon the ingestion of gluten [(1)]. So it is the result of both environmental (gluten) and genetic factors (carriers of HLA-DQ2 and DQ8 haplotypes). It is characterized by inflammation of the small-intestinal mucosa and numerous gastrointestinal and systemic manifestations. A duodenal biopsy with positive serology is the gold standard for the diagnosis of CD, communication of pathologist and gastroenterologists is essential for appropriate interpretation of duodenal biopsy [(2)]. The distribution of these two constituents can be used to identify the areas of the world at risk for gluten intolerance.

Celiac disease world geographical distribution seems to have followed the spread of wheat consumption in addition to the migratory flows of mankind. The classic clinical picture of pediatric celiac disease, which includes malnutrition, diarrhea, bloating, and abdominal pain, should be replaced with the more typical presentation of CD. The patient with CD is an asymptomatic school-aged child who belongs to a high-risk group. Despite improved awareness and screening protocols, many patients with CD may remain undiagnosed. It was recommended for the primary care physicians to implement screening programs in all high-risk populations, including first-degree family members of known patients with CD and patients with Down syndrome, Turner

syndrome, type 1 diabetes, thyroiditis, Addison disease, short stature, iron deficiency anemia, and unexplained elevation of aminotransferase levels. A high level of suspicion for CD should be entertained in other autoimmune disorders even if there are no apparent gastrointestinal symptoms [3]. Also, there has been some work looking to evaluate if other factors, such as gastrointestinal infection, surgery, or certain drugs, may be the trigger for development of CD [4].

Following the application of simple serological tests for the diagnosis of CD in the 1980s, it gradually became clear that the prevalence of CD in different countries in the Middle East, North Africa and India is almost the same as that in Western countries [5].

A significant change in diet habits, particularly in gluten consumption as well as in infant feeding patterns are probably the main factors that can account for these new trends in celiac disease epidemiology [6].

Europe historically was considered a geographical area at high frequency, with a prevalence of 1-2%, although it has been recently shown a similar prevalence in United States. It has been shown that CD is not exclusive of industrialized countries, but includes North Africa, Middle East and India with an incidence overlapping those of European countries. It has been shown that the Saharawi, an Algerian population has the highest prevalence of CD (nearly 6%) among all of the worldwide populations [7]. However, the diagnostic rate is mostly low in these countries due to low availability of diagnostic facilities and poor disease awareness. In the classical Anderson's textbook of Pediatric Gastroenterology first published in 1975, it was reported that 'The typical child with celiac disease, usually fair-haired, blue-eyed...'. The subsequent developments of CD epidemiology proved that this statement was incorrect as the highest prevalence of celiac disease in the world has been described in a black-eyed, black-haired mostly African population originally living in Western Sahara, the Saharawi, of Arab-Berber origin. Besides in a sample of 990 Saharawi children screened by anti-endomysium testing and intestinal biopsy, it was found a celiac disease prevalence of 5.6%, which is almost 5-fold higher than in most European countries.

The reasons behind such spiking frequency of CD are still unclear but could be primarily related to genetic factors given the high level of consanguinity of this population. The main susceptibility genotypes, HLA-DQ2 and -DQ8, exhibit one of the highest frequencies among them [8]. Also, it was found that the amount of gluten as well as gluten consumption patterns in early life have no impact on CD development before 6 years of age [9]. Other study on ethnic variations in duodenal villous atrophy consistent with CD in the United States mentioned that there were no significant differences in CD prevalence between Middle Eastern descent patients when compared with other Americans [10]. An Iranian study mentioned that CD in countries of Eastern Mediterranean Region Organization (EMRO) including North Africa and the Middle East are increasingly on the rise. In some studies, this disease has been diagnosed two to three times more in women than in men, besides the prevalence of celiac disease in Iran was similar or even higher than world-wide reported [11]. And in a systematic review and meta-analysis, it was found that celiac disease to be reported worldwide. The prevalence of celiac disease based on serologic test results was 1.4% and based on biopsy results was 0.7%. The prevalence values for celiac disease were 0.4% in South America, 0.5% in Africa and North America, 0.6% in Asia, and 0.8% in Europe and Oceania; the prevalence was higher in female vs male individuals (0.6% vs 0.4%; $P < 0.001$). The prevalence of celiac disease was significantly greater in children than adults (0.9% vs 0.5%; $P < 0.001$) and concluded that CD is a global disease and the global seroprevalence and prevalence of CD varies with sex, age, and geographic location. The prevalence of CD has increased over time from

0.6% in 1991 to 2000 to 0.8% between 2001 and 2016 [(12)].

So, CD clinical manifestations may vary with age, the duration and the extent of disease and multiple clinical studies showed that presentation with non-specific symptoms or no symptoms is as common in the Middle East as it is in Europe. The consumption of wheat has been the major primary food in these regions for many centuries and that the continuous and high level of exposure to its proteins induced some degree of immune tolerance, leading to milder symptoms, which are misdiagnosed as irritable bowel syndrome or unexplained gastrointestinal disorders. A high index of suspicion for CD should be maintained in all developing countries for patients who present with chronic diarrhea or iron deficiency anemia.

According to that, there is a need for population-based prevalence studies in many developing countries especially middle east to estimate the burden of CD properly.

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E-mail: dr.samaralshami1@gmail.com
dr.samaralshami1@colmed-alnahrain.edu.iq

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The Relation of Serum Omentin-1 Level with Insulin Resistance in Patients with Polycystic Ovary Syndrome and its Relation with Metformin Treatment

Jumana M. Kareem[1] *MSc,* **Zainab H. Hashim**[1] *PhD*, **Hala A. Almoayed**[2] *ABOG*

[1]Dept. of Physiology and Medical Physics, [2]Dept. of Gynecology and Obstetrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Polycystic ovary syndrome (PCOS) is still a matter of research looking for the true pathogenesis of this enigmatic syndrome. Although the majority of cases are familial, genetic studies have failed so far to identify the specific genes involved. The presentations of PCOS are heterogeneous and may change throughout the lifespan, starting from adolescence to post-menopausal age, and may have health impaction later in life. Omentin-1 is a fat depot-specific secretory protein produced by visceral stromal vascular cells. Recent studies showed that omentin-1 is correlated inversely with obesity and insulin resistance.

Objective To assess the serum omentin-1 concentration in PCOS women and the effect of metformin on omentin-1 level, to evaluate the role of omentin-1 on insulin resistance and hyperandrogenemia in PCOS women and to look for the correlation of omentin-1 with body mass index (BMI) in PCOS women.

Methods Eighty women involved in this study; 40 women with PCOS diagnosed according to Rotterdam ESHRE/ASRMS 2003 criteria and 40 apparently healthy women considered as the control group. The participants were allocated into six groups: "10 obese women with PCOS (BMI ≥30 kg/m^2, without metformin treatment)". "10 obese women with PCOS (BMI ≥30 kg/m^2, taking metformin)". "10 non-obese women with PCOS (BMI <30 kg/m^2, without metformin treatment)". "10 non-obese women with PCOS (BMI <30 kg/m^2, taking metformin)". "20 obese controls and 20 non-obese controls. Blood samples were taken from them for estimation of fasting blood glucose, insulin and omentin-1 levels. Hirsutism score was also evaluated according to Ferriman–Gallwey score.

Results There was a significant increase in omentin-1 in non-obese PCOS (taking metformin) (3.02±0.71) compared to obese PCOS (taking metformin) (1.59±1.48) (P value = 0.0132) and in PCO non-obese (taking metformin) (3.02±0.71) compared with control non-obese (1.96±1.65) (P value = 0.121). No significant correlation was found between serum omentin-1 level and insulin resistance as well as with hyperandrogenemia in any of the six study groups.

Conclusion Omentin-1 is found to be inversely related to body weight in PCOS women. Serum omentin-1 level has no effect on insulin resistance and hyperandrogenism states.

Keywords Polycystic ovaries PCOS, omentin, hyperandrogenemia, insulin resistance

Citation Kareem JM, Hashim ZH, Almoayed HA. The relation of serum omentin-1 level with insulin resistance in patients with polycystic ovary syndrome and its relation with metformin treatment. Iraqi JMS. 2017; Vol. 15(4): 327-338. doi: 10.22578/IJMS.15.4.2

List of abbreviations: ACTH = Adrenocorticotropic hormone, BMI = Body mass index, IGF = Insulin like growth factor, IR = Insulin resistance, LH = Luteinizing hormone, Mgpd = Mitochondrial glycerophosphate dehydrogenase, PCOS = Polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is the most common hormonal defect in child bearing women affecting about 7% of this population. The reproductive manifestation of PCOS consists of excess in androgen production and disordered gonadotropin secretion leading to menstrual irregularity, hirsutism and infertility [(1)]. In addition to these manifestations, PCOS has metabolic characteristics that include prominent defects in insulin action and β-cell function, defects that confer a substantially increased risk for glucose intolerance and type2 diabetes [(2)]. Obesity is a common finding in women with PCOS and between 40-80% of

women with this condition are reported to be overweight or obese [3].

Insulin resistance (IR) is the most conjoint finding in PCOS that is independent of obesity. Insulin-mediated glucose dumping, reflecting mainly insulin action on skeletal muscle is decreased by 35-40% in women with PCOS related to weight equivalent reproductively normal women [4]. This defect is independent of but considerably deteriorated by obesity. In contrast, hepatic IR, characterized by both excess postabsorptive glucose production and decreased sensitivity to insulin leading to suppression of endogenous glucose production, is existing only in obese women with PCOS related to control women of comparable body weight [2]. This synergistic deleterious influence of obesity and PCOS on endogenous glucose production may be a major factor in the pathogenesis of glucose intolerance [5].

Omentin is a fat depot-specific secretory protein produced by visceral stromal vascular cells, but not adipocytes. Omentin improved insulin-stimulated glucose transport and protein kinase B (Akt) phosphorylation in human subcutaneous and visceral adipocytes, proposing that omentin may improve insulin sensitivity [6]. Plasma omentin-1 levels, the major circulating isoform in human plasma, were related inversely with obesity and insulin resistance as determined by homeostasis model assessment yet correlated positively with adiponectin and HDL levels [7].

The objectives of this study were: to assess the serum omentin-1 concentration in PCOS women and the effect of Metformin on omentin-1 level, to evaluate the role of omentin-1 on IR and hyperandrogenemia in PCOS women and to study the correlation of omentin-1 with body mass index (BMI) in PCOS women.

Methods

This case control, nonrandomized study was conducted for evaluation of PCO patients who attended the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies. The study was approved by the Institution Review Board of the College of Medicine, Al-Nahrain University, and written consent was obtained from patients. The study was extended from November 2015 to March 2016.

Eighty women involved in the study who were arranged in groups: 40 infertile women with PCOS constituted this group as a patient group, which is subdivided into four subgroups:

1) Group 1a: comprised 10 obese (BMI ≥30 kg/m^2) PCOS patient taking metformin 500 mg twice daily for the last three months.
2) Group 1b: included 10 obese (BMI ≥30 kg/m^2) PCOS patient not taking metformin for the last 3 months.
3) Group 1c: comprised ten non-obese (BMI <30 kg/m^2) PCOS patient taking metformin 500 mg twice daily for the last 3 months.
4) Group 1d: included ten non-obese (BMI <30 kg/m^2) PCOS patient not taking metformin for the last 3 months.

Forty apparently normal women considered as a control group, who were free from PCOS; they were further subdivided into two groups depending on the BMI into:

1) Group 2a: consist of twenty obese subjects with BMI ≥30 kg/m^2
2) Group 2b: encompassed twenty non-obese subjects with BMI <30 kg/m^2.

Inclusion criteria

1. Age between 18-35 years.
2. Have no other endocrine disease.

Exclusion Critera

1. Hyperprolactinemia
2. History of type II diabetes mellitus
3. Women with history of gestational diabetes mellitus

The following features were noted: menstrual history; presence of acne; hirsutism; BMI; a diagnosis of polycystic ovaries on ultrasound was based on the presence of 12 or more follicles measuring 2-9 mm and/or ovarian volume measuring >10 cm^3 [8]. The cases were examined by consultant physician in the infertility institute. Hirsutism is based on the

visual scoring method described by modified Ferriman and Gallwey scale. Nine body areas examined: upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, the upper arms and the thighs, hair growth is rated from 0 (no growth of terminal hair) to 4 (extensive hair growth) in each of the nine locations. A patient's score may therefore range from a minimum score of 0 to a maximum score of 36 cut-off value ≥6-8 [9].

The following parameters and biochemical measurements were done for the all subjects included in this study:

BMI assessment

it is equal to mass (kg)/ (height (m))^2. Obese (BMI ≥30 kg/m^2) and non-obese (BMI <30 kg/m^2).

Biochemical tests

Fasting blood glucose (FBG)

The examined individuals should be fasting for at least 12 hours prior to the test. three ml of venous blood samples were aspirated transferred into clean, plain tubes and centrifuged within 30 minutes of collection. Then the serum from all blood samples were separated; sugar was measured using enzyme colorimetric methods and the rest were stored at -20 °C.

Hormonal assay

1) Serum omentin.
2) Serum insulin.

Omentin was measured by using an omentin enzyme immunoassay or ELISA kit (Elabscience), according to the manufacturer's instructions.

Insulin hormone was measured by using an insulin enzyme immunoassay or ELISA kit (Calbio tech, Insulin Elisa), according to the manufacturer's instructions.

Homa insulin resistance also measured, which defined as fasting insulin (µIU/L) x Fasting glucose (mg/dL) / 405. HOMA score was < 3 considered normal, between 3 and 5 moderate IR and >5 severe IR [10].

Results

Omentin-1 level of group 1a was significantly lower than that of group 1c (P value = 0.01). On the contrary insulin level show significant increase in group 1a in comparison with group 1c (P value = 0.002), moreover, IR was significantly increased in group 1a in comparison with group 1c (P value = 0.001) (Table 1). While no significant differences were noticed in FBS level and hirsutism score (Tables 1 and 2)

Omentin-1 level of group 1a was significantly lower than that of group 1c (P value = 0.01). On the contrary insulin level show significant increase in group 1a in comparison with group1c (P value = 0.002), moreover, IR was significantly increased in group 1a in comparison with group 1c (P value = 0.001), (Table 3).

No significant differences were noticed in FBS level and hirsutism score (Tables 3 and 4).

Table (5) showed that insulin level, insulin resistance and FBS were significantly decreased in the control group as a whole when compared to PCOS patient without metformin treatment (P=0.02; 0.01; 0.02, respectively). On the contrary omentin-1 level was not different between the two groups.

Moreover, hirsutism score of PCOS group showed a significant increase in comparison to control group (P value = 0.01), furthermore those ≥ 6 in same group also showed a significant increase in comparison to control group (P value = 0.01) (Table 6).

Table 1. Comparison between patients with polycystic ovary syndrome without metformin treatment groups by unpaired t-test

Parameter	PCOS patients without metformin treatment		P value
	Obese N=10	Non-obese N=10	
Body weight (kg)	92.5±10.32	62.1±11.53	< 0.0001
Fasting blood sugar (mg/dl)	92.7±6.2	88.6±8.81	0.2444
Serum Insulin (µIU/ml)	20.38±10.55	9.29±6.73	0.0118
Serum Omentin (ng/ml)	1.61±0.84	2.52±1.39	0.0929
Insulin resistance	4.64±2.39	2.06±1.46	0.0093

The data presented in mean±SD, PCOS = Polycystic ovary syndrome

Table 2. Comparison of hirsutism between obese and non-obese patients with polycystic ovary syndrome by Fisher exact test

Parameter	PCOS patients without metformin treatment		P value
	Obese N=10	Non-obese N=10	
< 6	7 (70)	9 (90)	0.582
≥ 6	3 (30)	1 (10)	

The data presented with number and percentage, PCOS= polycystic ovary syndrome

Table 3. Comparison between obese and non-obese PCOS with metformin treatment groups by unpaired t-test

Parameter	PCOS patients with metformin treatment		P value
	Obese N=10	Non-obese N=10	
Body weight (kg)	86.8±9.86	67.7±10.09	0.0004
Fasting blood sugar (mg/dl)	95.7±4.08	90.8±6.83	0.0672
Serum Insulin (µIU/ml)	9.66±5.88	2.77±1.44	0.0021
Serum Omentin (ng/ml)	1.59±1.48	3.02±0.71	0.0132
Insulin resistance	2.27±1.34	0.61±0.29	0.0012

The data presented in mean±SD, PCOS = Polycystic ovary syndrome

Table 4. Comparison of hirsutism between obese and non-obese PCOS on metformin treatment by Fisher exact test

Parameter	PCOS patients with metformin treatment		P value
	Obese N=10	Non-obese N=10	
< 6	6 (60)	6 (60)	1.000
≥ 6	4 (40)	4 (40)	

The data presented with number and percentage, PCOS= polycystic ovary syndrome

Table 5. Comparison between control group and PCOS group without metformin treatment by unpaired t-test

Parameter	Control N=40	PCOS patients without metformin treatment N=20	P value
Body weight (kg)	72.08±15.28	77.3±18.88	0.2909
Fasting blood sugar (mg/dl)	84.73±10.62	90.65±7.71	0.0173
Serum Insulin (µIU/ml)	8.38±7.28	14.84±10.32	0.0183
Serum Omentin (ng/ml)	1.73±1.49	2.07±1.21	0.3508
Insulin resistance	1.78±1.55	3.35±2.33	0.0111

The data presented in mean±SD, PCOS = Polycystic ovary syndrome

Table 6. Comparison of hirsutism between obese and non-obese PCOS on metformin treatment by Fisher exact test

Parameter	Control N=40	PCOS patients without metformin treatment N=20	P value
< 6	40 (100)	16 (80)	0.010
≥ 6	0 (0)	4 (20)	

The data presented with number and percentage, PCOS= polycystic ovary syndrome

There was significant decrease in FBS of control group in comparison with PCOS group (on metformin treatment) (P value = 0.0002). No significant differences were noticed in omentin, insulin levels and insulin resistance, BMI (Table 7).

Hirsutism score of PCOS group on metformin treatment < 6 showed a significant increase in comparison to control group (P value =0.001). Furthermore, those ≥ 6 in same group also showed a significant increase (8 in comparison to control group (P value = 0.001) (Table 8).

The insulin level and IR were significantly increased in PCOS patients without metformin treatment versus those on metformin treatment (P=0.002; P=0.003, respectively), (Table 9).

No significant differences were noticed in FBS, omentin level, BMI and hirsutism score (Tables 9 and 10).

Table 7. Comparison between control group and PCOS group with metformin treatment by unpaired t-test

Parameter	Control N=40	PCOS patients with metformin treatment N=20	P value
Body weight (kg)	72.08±15.28	77.25±13.8	0.1937
Fasting blood sugar (mg/dl)	84.73±10.62	93.25±6.03	0.0002
Serum Insulin (µIU/ml)	8.38±7.28	6.21±5.47	0.2027
Serum Omentin (ng/ml)	1.73±1.49	2.31±1.35	0.1397
Insulin resistance	1.78±1.55	1.44±1.27	0.3729

The data presented in mean±SD, PCOS = Polycystic ovary syndrome

Table 8. Comparison of hirsutism between control group and PCOS group with metformin treatment by Fisher exact test

Parameter	Control N=40	PCOS patients with metformin treatment N=20	P value
< 6	40 (100)	12 (60)	0.001
≥ 6	0 (0)	8 (40)	

The data presented with number and percentage, PCOS= polycystic ovary syndrome

Table 9. Comparison between PCOS patients on metformin and those without metformin treatment by unpaired t-test

Parameter	PCOS patients		P value
	Without metformin treatment N=20	With metformin treatment N=20	
Body weight (kg)	77.3±18.88	77.25±13.8	0.9924
Fasting blood sugar (mg/dl)	90.65±7.71	93.25±6.03	0.2420
Serum Insulin (µIU/ml)	14.84±10.32	6.21±5.47	0.0021
Serum Omentin (ng/ml)	2.07±1.21	2.31±1.35	0.5604
Insulin resistance	3.35±2.33	1.44±1.27	0.0027

The data presented in mean±SD, PCOS = Polycystic ovary syndrome

Table 10. Comparison of hirsutism between PCOS group without metformin treatment and PCOS group with metformin treatment by Fisher exact test

Parameter	PCOS patients		P value
	Without metformin treatment N=20	With metformin treatment N=20	
< 6	16 (80)	12 (60)	0.301
≥ 6	4 (20)	8 (40)	

The data presented with number and percentage, PCOS= polycystic ovary syndrome

Insulin level was highly significantly decreased in group 2a in comparison with group 1b (P value =0.02). IR of group 2a was significantly lower than that of group 1b (P value = 0.01). No significant differences were noticed in, FBS, omentin level (Table 11).

Hirsutism score of group 1b < 6 showed a significant increase in comparison to group 2a (P value = 0.03), furthermore, those ≥ 6 in same group also showed a significant increase in comparison to group 2a (P value =0.03) (Table (12).

Table 11. Comparison between obese control group and obese PCOS group without metformin treatment by unpaired t-test

Parameter	Obese Control N=20	Obese PCOS patients without metformin treatment N=10	P value
Body weight (kg)	83.75±10.47	92.5±10.32	0.0426
Fasting blood sugar (mg/dl)	86.5±11.56	92.7±6.2	0.0664
Serum Insulin (µIU/ml)	10.38±8.01	20.38±10.55	0.019
Serum Omentin (ng/ml)	1.5±1.32	1.61±0.84	0.7702
Insulin resistance	2.27±1.78	4.64±2.39	0.0148

The data presented in mean±SD, PCOS = Polycystic ovary syndrome

Table 12. Comparison of hirsutism between PCOS group without metformin treatment and PCOS group with metformin treatment by Fisher exact test

Parameter	Obese Control N=20	Obese PCOS patients without metformin treatment N=10	P value
< 6	20 (100)	7 (70)	0.030
≥ 6	0 (0)	3 (30)	

The data presented with number and percentage, PCOS= polycystic ovary syndrome

FBS of group 2a was significantly lower than that of group 1a (P value =0.003) (Table 13). No significant differences were noticed in insulin, omentin, BMI and insulin resistance.
Hirsutism score of group 1a < 6 showed a significant increase in comparison to control group 2a (P value =0.008), furthermore those ≥ 6 in same group also showed a significant increase in comparison to control group (P value <0.008) (Table 14).

Table 13. Comparison between obese control group and obese PCOS group with metformin treatment by unpaired t-test

Parameter	Obese Control N=20	Obese PCOS patients with metformin treatment N=10	P value
Body weight (kg)	83.75±10.47	86.8±9.86	0.4437
Fasting blood sugar (mg/dl)	86.5±11.56	95.7±4.08	0.0037
Serum Insulin (µIU/ml)	10.38±8.01	9.66±5.88	0.7827
Serum Omentin (ng/ml)	1.5±1.32	1.59±1.48	0.8657
Insulin resistance	2.27±1.78	2.27±1.34	0.9982

The data presented in mean±SD, PCOS = Polycystic ovary syndrome

Table 14. Comparison of hirsutism between obese control group and obese PCOS group with metformin treatment by Fisher exact test

Parameter	Obese Control N=20	Obese PCOS patients with metformin treatment N=10	P value
< 6	20 (100)	6 (60)	0.008
≥ 6	0 (0)	4 (40)	

The data presented with number and percentage, PCOS= polycystic ovary syndrome

Omentin level in group 2b was significantly lower than that of group 1c (P value = 0.02). Insulin level show highly significant increase group 2b in comparison group1c (P value = 0.02). Insulin resistance of group 2b was also significantly higher than Insulin resistance of group1c (P value = 0.02). On the reverse, FBS shows significant decrease group2b in comparison with group1c (P value = 0.02), (Table 15). No significant difference was noticed in BMI. Moreover, hirsutism score of group1c < 6 showed a significant increase in comparison to group2b (P value < 0.008), furthermore those ≥ 6 in same group also showed a significant increase in comparison to group2b (P value < 0.008) (Table 16).
Table (17) illustrates a significant negative correlation between omentin and BMI in patients of group1a and 1c (r = -0.472, P value = 0.035).
There was no significant correlation in fasting blood sugar, serum insulin and insulin resistance and hirsutism score among these groups.

Table 15. Comparison between non-obese control group and non-obese PCOS group on metformin treatment by unpaired t-test

Parameter	Non-obese Control N=20	Non-obese PCOS patients with metformin treatment N=10	P value
Body weight (kg)	60.4±9.09	67.7±10.09	0.071
Fasting blood sugar (mg/dl)	82.95±9.55	90.8±6.83	0.0162
Serum Insulin (µIU/ml)	6.39±6.03	2.77±1.44	0.0183
Serum Omentin (ng/ml)	1.96±1.65	3.02±0.71	0.021
Insulin resistance	1.29±1.13	0.61±0.29	0.0192

The data presented in mean±SD, PCOS = Polycystic ovary syndrome

Table 16. Comparison of hirsutism score between non-obese control group and non-obese PCOS group on metformin treatment by Fisher exact test

Parameter	Obese Control N=20	Non-obese PCOS patients with metformin treatment N=10	P value
< 6	20 (100)	6 (60)	0.008
≥ 6	0 (0)	4 (40)	

The data presented with number and percentage, PCOS= polycystic ovary syndrome

Table 17. Correlation of omentin with body mass index, Fasting blood Sugar, Serum Insulin, Insulin resistance and Hirsutism score

Parameter	Control N=40		PCOS without metformin treatment N=20		PCOS on metformin treatment) N=20	
	r	p	r	p	r	p
BMI (kg/m^2)	-0.090	0.582	-0.270	0.250	-0.472	0.035
Fasting blood Sugar	-0.029	0.859	0.291	0.213	-0.441	0.052
Serum Insulin	0.176	0.278	-0.118	0.622	0.053	0.823
Insulin resistance	0.129	0.429	-0.080	0.739	0.014	0.952
Hirsutism score	-	-	0.237	0.315	0.163	0.494

PCOS= polycystic ovary syndrome

Discussion

Omentin-1 lipoprotein is a newly discovered adipokine mediator as it is released from visceral stromal mesenchymal vascular tissue fatty and non-fatty cells and its release in plasma is act as a bi-product of lipid metabolism (anabolic and catabolic pathways) as in lipid peroxidation. It has prospect values

in management of certain endocrine disorders like DM type2 and polycystic ovary and its relation to insulin resistance level in patients with polycystic ovary syndrome [11].

The state of hyperinsulinemia may itself contribute to obesity by the anabolic effect of insulin on fat metabolism through adipogenesis via increased uptake of glucose into adipocytes, which eventually leading to the production of triglycerides and inhibition of hormone sensitive lipase [12]. While other researchers found that IR in PCOS may lead to low energy expenditure, where those women appear to have significantly lower basal metabolic rate than do age- and BMI-matched controls (1446 kcal/day versus 1841 kcal/day). Although many patients with PCOS have IR independent of obesity, the obesity worsens underlying IR and insulin resistance-associated reproductive and metabolic effects [13].

In the present study, there is significant difference between the hirsutism score in PCOS patient and control subjects. This is agreed with Sirmans et al. [14] who reported that PCOS is a common heterogeneous endocrine disorder characterized by irregular menses, hyperandrogenism, and hirsutism in addition to polycystic ovaries, suggesting that a primary defect in androgen metabolism is the intrinsic, major factor in the pathogenesis of PCOS [15].

Current study found that there is no significant difference in serum omentin-1 level between non-obese PCO without metformin treatment in comparison with non-obese control group, this is in agreement with Akbarzadeh et al. [16] who proved that PCOS is not a determinant of decreased omentin plasma level may be due to high androgen level and IR as warning signs of PCOS. While in contradiction with Yang et al. [17] who reported significant decrease in plasma omentin level of non-obese PCO in comparison with healthy control, this may be due to larger sized sample (n=153 healthy group, 114 PCOS individuals) contributes to this controversy [16].

Furthermore, there is no significant difference in serum omentin-1 level between obese PCO without metformin treatment in comparison with obese control group, this concept in disagreement with Mahde et al. [18] who revealed that there is significant difference between obese PCO and control group, the differences in BMI of control group between their study (29.56 ± 2.12) and ours (33.38±3.7) may explain this discrepancy.

Metformin's main action is to decrease the overproduction of glucose by the liver, a common problem in prediabetes and type 2 diabetes. The action of metformin helps lower blood sugar levels particularly during the night to keep fasting glucose levels under control, but it also helps control blood glucose throughout the day. Metformin also increases the uptake of glucose by your muscles. Overall, metformin decreases IR and improves insulin sensitivity, thereby helping the insulin your body still makes work more effectively [19,20].

One of the main metabolic features of metformin is its ability to reduce hepatic glucose production [21]. A recent study suggested that inhibition of mitochondrial glycerophosphate dehydrogenase (mGPD), a critical enzyme in the glycerophosphate shuttle, could be the primary mechanism of metformin-induced inhibition of gluconeogenesis [22,23].

Other studies have shown that the intestines play a significant role in the glucose-lowering effect of metformin by facilitating uptake and utilization of glucose [24].

This study concluded that serum omentin-1 level is decreased remarkably with increasing body weight in PCOS. Also, non-obese PCOS respond better to metformin treatment than obese PCOS in enhancing insulin sensitivity and in increasing serum omentin-1 concentration. Furthermore, this study showed no effect of serum omentin-1 level on insulin resistance and hyperandrogenism.

This study recommends to do a research on larger sized groups to ensure more precise prediction of changes in different variables. Also recommends doing a paired study (before and after taking metformin) is advisable to be in account than unpaired study in further

researches. Longer duration (>3 months) of metformin treatment is as well recommended to achieve better results regarding omentin-1 effect in PCOS patients.

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Authors Contribution:
Dr. Kareem conducted the study, collected the data and performed the statistical analysis and drafting the manuscript. Dr. Hashim contributed in the designing, organization and finalization of manuscript. Dr. Almoayed: referring the PCOS cases.

Conflict of interest
The authors declare no conflict of interest.

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Correspondence to Dr. Jumana M. Kareem
E-mail: jumana86.jm@gmail.com
dr_jumana.jm@colmed-alnahrain.edu.iq
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The Risk Factors and Frequency of Congenital Anomalies in Neonates Born after Assisted Reproductive Technique in Baghdad

Deia K. Khalaf *FICMS*

Dept. of Pediatrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Assisted reproductive technique (ART) has helped couples all over the world. There have already been over 3,500,000 births resulting from ART, and with falling fertility in some countries.

Objective To identify the frequency and types of congenital anomalies among neonates born after ART, and to identify the probable fetal and maternal predisposing factors that may associated with these congenital anormalities and neonatal complications.

Methods This prospective study was performed in the Neonatal Intensive Care Unit in 3 teaching hospitals in Baghdad, from 1st day of January to day 31 of December 2015, and 306 live birth neonates were delivered by ART, and evaluated by the researcher and his residence pediatricians' doctors, and other congenital anomalies were assessed by ultrasonography, x-ray and echocardiography. Information about each neonate were taken from the records and families which includes: gestational age (term ≥ 37 week and preterm < 37 week) no post-term case were reported, body-weight (≥ 2.5 kg and < 2.5 kg), sex, system affected, age of the parents, consanguinity, residence, job of the parents, level of education, health condition of the parents, causes of infertility, any family history of congenital anomalies, death in the family. Exclusion criteria included mothers' age above 40, any maternal chronic diseases and chronic drugs taken. Congenital anomalies were classified into systems according to WHO recommendation.

Results Three hundred and six neonates were delivered, from which, 30 (10%) had congenital anomalies with male to female ratio (1.2:1), (20 (67%) twins and 10 (33%) were singletons), a significant association between congenital anomalies in ART products and male sex, consanguinity, and gestational age, as the p-value is significant (< 0.05), and the most common system affected was the gastro-intestinal tract (3%), but there was no significant association with body-weight.

Conclusion The ART born neonates are more prone for congenital anomalies. Gastrointestinal anomalies, especially esophageal atresia, are the commonest type of congenital anomalies followed by neurological anomalies. Male sex, consanguinity, and gestational age are significant risk factors for congenital anomalies. While body weight had no significant association with congenital anomalies.

Keywords Assisted reproductive technique (ART), intensive care unit, gastro-intestinal tract.

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List of abbreviations: ART = Assisted reproductive technique, IVF = In vitro fertilization

Introduction

The first successful human in vitro fertilization (IVF) attempt resulted in the 1978 delivery of Louise Brown in England and is considered the beginning of a new era for the treatment of infertility [1]. Birth defects (congenital anomalies) according to World Health Organization are structural, functional, and/or biochemical-molecular defects present at birth [2]. Early intrauterine period during 3rd-8th weeks of gestation is the vital period of life for the normal development of organs and organ system or organogenesis

[3]. On most health indicators, children born after ovulation induction (OI) seem to perform worse compared with spontaneously conceived children [4] or defined more widely to include functional disturbance as a defect, any irreversible condition exiting in a child before birth [5,6]. Birth defects are still the leading cause of perinatal mortality and childhood disability in developed countries [7]. However, birth defects in the developing world are largely underreported by deficiencies in diagnostic capabilities and lack of reliability of medical records and health statistics [8]. Factors may increase the risk of birth defects include the relatively advanced age of infertile couples, the underlying cause of their infertility, the medications used to induce ovulation or to maintain the pregnancy in the early stages, and factors associated with the procedures themselves, such as the freezing and thawing of embryos, the potential for polyspermic fertilization, and the delayed fertilization of the oocyte [9].

This study aims to identify the frequency and types of congenital anomalies among Assisted Reproductive Technologies (ART) born infants and to assess the probable fetal and maternal predisposing factors that may associated with congenital abnormalities and neonatal complications in ART.

Methods

This is a prospective study performed in Al-Imamein Al-kadhimein Medical City, Baghdad Teaching Hospital, and Al-Yarmook Teaching Hospital at the Neonatal Intensive Care Unit in a period from the 1st of January 2015 to the 31st of December 2015 during the first week of the neonate's age.

The total number of delivery from ART was 306 live births, from these deliveries, there were 30 (10%) neonates have obvious congenital anomalies, (20 twins and 10 as singletons) evaluated in the Neonatal Unit by researcher and his residence pediatricians' doctors, and other congenital anomalies were assessed by ultrasonography, x-ray and echocardiography study. Information list for each newborn, taking the detail from the neonatal records and families which includes: gestational age (the neonates were classified into term $\geq$ 37 week and preterm < 37 weeks); no post-term was reported, bodyweight (the neonates were classified into $\geq$ 2.5 kg and < 2.5 kg), sex, system affected, age of the parents, consanguinity, residence, job of the parents, level of their education, health condition of the parents, causes of infertility, any family history of congenital anomalies and if there is death in the family. Exclusion criteria included mother's age above 40, any maternal chronic diseases or drugs taken. Congenital anomalies were classified into systems according to World Health Organization recommendation [10]. We compare the different variables with control cases of normal conception (1000 cases) that has been taken at the Neonatal Unit from them 10 cases have congenital anomalies with matching age group (20-40) years for the mothers and gravidity (primigravida).

Statistical analysis was done using SPSS version 20 software program, chi-square test was used and a p-value <0.05 was considered significant.

Results

The study included 306 neonates (168 males and 138 females) with male to female ratio (1.2:1), from which, 30 cases (18 males and 12 females) had congenital malformation, (20 twins and 10 as singletons), with frequency of 10%, as shown in table (1), while in normal conception the frequency (1%) and p value (< 0.001), which is significant.

Table 1. Distribution of neonates with congenital malformation in both groups according to their sex

Sex	Total Live Birth By ART*	Congenital Malformation No. (%)	Total Live Birth By N C**	Congenital Malformation No. (%)	P Value
Male	168	18 (6%)	514	6 (0.6%)	<0.001
Female	138	12 (4%)	486	4 (0.4%)	
Total	306	30 (10%)	1000	10 (1 %)	

ART*= Assisted reproductive technique. N C**= Normal conception

Gastro-intestinal tract (GIT) anomalies was the commonest system 9 (3%) affected with (50%, was esophageal atresia, 33. %, imperforated anus, and 7%, of diaphragmatic hernia), followed by Central nervous system (CNS) anomalies 8 (2.7%), (40% hydrocephaly, 40% spinabifida, 20% anencephaly). Cardiovascular system (CVS) 6 (1.9%), (50%, ventricular septal defect (VSD)+ patent ductus arteriosus (PDA), 25% PDA, 25% dilated cardiomyopathy). Musculoskeletal system 4 (1.4%), (75% developmental dysplasia of the hip, 25% high arched palate), while the renal system was the least one 3 (0.9%), (67% polycystic kidney disease, 0.33% hydronephrosis), and with comparison with normal conception group the p-value=0.004 as in table 2.

Table 2. The relationship between congenital anomalies and system affected

System affected	No. of cases ART*	male No.	Female No.	%	No. of cases N C**	Male No.	Female No.	%
Gastrointestinal	9	6	3	3	2	2	0	0.2
Central nervous system	8	6	2	2.7	3	2	1	0.3
Cardiovascular	6	2	4	1.9	2	1	1	0.2
Musculoskeletal	4	2	2	1.4	1	0	1	0.1
Renal	3	2	1	0.9	2	2	0	0.2
P value				0.004				

ART*= Assisted reproductive technique. N C**= normal conception

Consanguinity showed significant association with congenital anomalies 70%, and p-value = (0.0228), as shown in table 3.

Gestational age had a significant association with congenital anomalies the p-value (0.002). As in table 4.

Table 3. The relationship between consanguinity and congenital anomalies

System affected	ART* No.	Consanguinity +ve	Consanguinity -ve	NC** No.	Consanguinity +ve	Consanguinity -ve
Central nervous system	8	6	2	3	2	1
Cardiovascular	6	4	2	2	2	0
Renal	3	2	1	2	1	1
Gastrointestinal	9	6	3	2	1	1
Musculoskeletal	4	3	1	1	0	1
Total No.	30	21 (70%)	9 (30%)	10	6 (60%)	4 (40%)
P value			0.0228			

ART*= Assisted reproductive technique. N C**= normal conception

Table 4. The relationship between gestational age and system affected

System affected	Total no. of ART*	Term ≥ 37 weeks	Preterm < 37 weeks	No. of NC**	Term ≥ 37 weeks	Preterm < 37 weeks
Central nervous system	8	3	5	3	1	2
Cardiovascular	6	2	4	2	0	2
Renal	3	1	2	2	2	0
Gastrointestinal	9	3	6	2	1	1
Musculoskeletal	4	3	1	1	0	1
P-value			0.002			

ART*= Assisted reproductive technique. N C**= normal conception

Body weight had no significant association with congenital anomalies, as the p= value (0.6446) as in table 5.

Discussion

The current study showed that the frequency of congenital malformation among the ART born neonates 10%, which was in agreement with Allen et al. study done in Canada (9%) [11], but higher than study done by Mozafari Kermani et al. (7%) [12], while in general population (2-3%) [13,14], which similar to study in Finland (5.5-6.6%) [15], Netherlands (2.3%,3.7%) [16,17], England (4.8%) [18], Australia (4.3%) [19], Sweden (5%) [20], Germany (8.6%) [21] and Australia (8.9%) [22]. The variation in results probably because of small mass and short duration of the current study.

Current study shows a higher predominance of male gender (males 5.88%: females 3.92%), which in accordance With Iranian study done by Movafagh et al. [23].

Table 5. The relation between the body Weight and system affected

System affected	Total no. of ART*	Body weight ≥ 2.5kg	Body weight < 2.5kg	No. of NC**	Body weight ≥ 2.5kg	Body weight < 2.5kg
Central nervous system	8	5	3	3	1	2
Cardiovascular	6	2	4	2	0	2
Renal	3	2	1	2	1	1
Gastrointestinal	9	5	4	2	2	0
Musculoskeletal	4	2	2	1	0	1
P-value	0.6446					

ART*= Assisted reproductive technique. N C**= normal conception

The current study showed predominance of GIT anomalies 2.94% followed by CNS anomalies 2.45%, CVS anomalies 1.96%. Musculoskeletal 1.47% then Renal 0.98%. A study done by Allen et al. showed the predominance of musculoskeletal 3.3%, Renal 2.6%, CVS 1.8%, GIT 0.6% and CNS 0.4% [11], this difference may related to study mass or regional variation and may be due to genetic factor.

Consanguinity among neonates was found in 21 (70%), which in accordance with study by Khatemi and Mamorri (67%) [24], and Chaturvedi and Banerjee (71%) [25]. The current study showed a significant statistical association between gestational age and congenital anomalies, which was in agreement with Davies et al. [26].

This study recommended that the ART neonates have high frequency of congenital anomalies, with high frequency in males than females. Gastrointestinal anomalies, especially esophageal atresia was the commonest type of congenital anomalies followed by neurological anomalies. Consanguinity and gestational age had significant association with congenital anomalies in ART neonates. There is no significant association between body weight and congenital anomalies in ART neonates.

This study recommends that ART contributes to significant risk of congenital malformation and may be more pronounced for multiple pregnancies so accurate counseling for parents considering ART and multidisciplinary coordination of care prior to delivery are warranted. Discussion of options for prenatal screening for congenital structural abnormalities in pregnancies achieved by ART is recommended, including appropriate use of Biochemical and sonographic screening. Further scientific research is needed to determine the relation between specific type of ART (as far as there are different methods of ART), and congenital abnormalities.

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Conflict of interest

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E-mail: deiaasdy1960@gmail.com
deiaasdy60@colmed-alnahrain.edu.iq
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Serum Levels of Glycodelin A and Soluble Intracellular Adhesion Molecule-1 as Biomarkers for Endometriosis

Hadeel A. Draj[1] *MSc,* **Ahmed A. Abbas**[1] *PhD*, **Thurya H. Abdullah** [2] *ABOG, FICOG*

[1]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Al-Imamein Al-Kadhimein Medical City, Baghdad, Iraq

Abstract

Background Endometriosis is a benign chronic disease, characterized by the presence and proliferation of functional endometrial gland and stroma outside the uterine cavity.

Objective To evaluate the usefulness of intracellular adhesion molecule-1 (ICAM-1) and Glycodelin (Gd) as a biomarker for the diagnosis of endometriosis and to help in detection of various stages of endometriosis.

Methods Forty-four patients with endometriosis and 35 apparently healthy women as control were enrolled in this study from November 2015 to April 2016. All individuals were subjected to blood sampling for measuring their serum ICAM-1 and Gd A level by using enzyme linked immune sorbent assay technique.

Results The current study revealed significantly higher serum levels of ICAM-1 and Gd in patients group in comparison with healthy control. In addition, the sensitivity and specificity for ICAM-1 and Gd A in serum were 61%/ 66%, 76%/ 85% respectively.

Conclusion The ICAM-1 and Gd A level in serum may be useful as noninvasive test for diagnosis of endometriosis in all stages.

Keywords Endometriosis, ICAM-1, Glycodelin

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List of abbreviations: ELISA = Enzyme linked immune sorbent assay, Gd = Glycodelin, ICAM-1 = Intracellular adhesion molecule-1, LAF-1 = Lymphocyte function association antigen-1

Introduction

Endometriosisis a chronic gynecologic disorder defined by the kind of tissue lines the uterus known endometrial tissue also grows in other part of the body [(1)]. The disease exhibits a broad spectrum of clinical signs and symptoms and often present vexing clinical management problems for women and their physicians [(2)]. Soluble intercellular adhesion molecule-1 (sICAM-1) represents a circulating form of ICAM-1 that is constitutively expressed or is inducible on the cell surface of different cell lines. It serves as a counter-receptor for the lymphocyte function-associated antigen (LFA-1). Interaction between sICAM-1, present on endothelial cells and LFA-1 facilitates leukocyte adhesion and migration across the endothelium. sICAM-1 and its circulating form have been implicated in the development of any number of diseases [(3)]. Levels of the sICAM-I have been suggested to elevate during early stages of endometriosis (I-II) and decrease at stage (III-IV) [(4)]. Glycodelin (Gd), an endometrium-derived protein with known angiogenic, immunosuppressive,

contraceptive effects, could contribute to the development of endometriosis and endometriosis-associated infertility [5]. Moreover, Gd is not only produce in the glandular epithelium of secretary endometrium [6,7], but also is shed from endometriotic lesion into the peritoneal fluid and serum. Increase plasma glycodelin levels have been observe in patients with endometriosis [8].

The biomarkers that are simple to measure could help clinician to diagnose or at least exclude endometriosis; it might also allow monitor the treatment effect. A precise blood or urine test could avoid the need for an invasive procedure (9).

The current study aimed to evaluate the usefulness of ICAM-1 and Gd A as a biomarker for the diagnosis of endometriosis and to help in detection of various stages of endometriosis.

Methods

Forty-four women with endometriosis diagnosed by laparotomy or laparoscopy investigations confirmed by histopathological report were involved in the current study, their age range from 18 to 48 years. They were collected from Aum Albanin Center for Infertility at Al-Imamein Al-Kadhimein Medical City and from Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University from November 2015 to April 2016. They were classified by a gynecologist according to the American Fertility Society (now the American Society for Reproductive Medicine; ASRAM) into different stages (minimal, mild, moderate and severe endometriosis).

In addition, the current study included 35 apparently healthy volunteers whose ages were matched with patients group, were considered as control. All of these healthy women were a symptomatic with regular menstrual cycle and fertile.

Five ml of venous blood was drawn from each patient and control. Blood sample was collected in glasses gel tubes for serum separation. The serum sample was divided in to aliquots, and kept in deep freeze till used. The measurement of serum sICAM-1 and Gd was done by ELISA technique according to instructions manual by Human Company/ Germany in Education Labs / Medical City. Ethical approval and informed consent were obtained from each participant in this study according to the declaration of Helsinki-ethical agreement, it was obtained from the Institutional Review Board of College of Medicine, Al-Nahrain University.

Results

The age of patients with endometriosis ranged between 18 to 48 years with a mean age 30.54±8.39 years while the mean age of healthy women was 33.92±14.7 as shown in table (1).

Table 1. Comparison between patients group and control group according to the age by unpaired t test

Parameter	Patients N=44 Mean±SD	Control N=35 Mean ±SD	P value
Age (yr)	30.54+8.39	33.92+14.7	0.192

The current study revealed significantly higher serum levels of Gd A and sICAM-1 in patients group in comparison with control group (Table 2).

Regarding ELISA technique was showed acceptable sensitivity and specificity of sICAM-1 and Gd A were (61%/66%) and (76%/85%) respectively (Table 3).

The comparison of serum level of parameters (ICAM-1 and Gd) according to stages of disease showed no important difference among patients group (Table 4).

Table 2. Comparison between patients and control groups according to serum level of sICAM-1 and Glycodelin by Mann Whitney U test

Parameters	Patients N=44 Median (Range)	Control N=35 Median (Range)	P value
sICAM1 (ng/ml)	98 (65-191)	92.5 (84-113)	0.005
Glycodelin (ng/ml)	135 (60-240)	92 (0-160)	< 0.001

Table 3. Sensitivity and specificity percentage of ELISA test regarding ICAM-1 and Glycodelin

Parameters	Sensitivity	Specificity	Cutoff value
ICAM1 (ng/ml)	61.9%	66.7%	95.5
Glycodelin (ng/ml)	76.2%	85.7%	99.5

Table 4. Comparison the level of parameters according to stage of Endometriosis in patients group

Parameters	Stage 1 N=4 Mean±SD	Stage 2 N=9 Mean±SD	Stage 3 N=13 Mean±SD	Stage 4 N=18 Mean±SD	P value
ICAM1 (ng/ml)	110.14±36.15	91.63±11.84	108.45±11.38	99.06±9.38	0.113
Glycodelin (ng/ml)	163.67±60.73	120.38±29.49	125.69±34.36	136.67±46.39	0.249

Discussion

Mean levels of sICAM-1 appeared significantly increase in patients with endometriosis with sensitivity and specificity (61-66%) respectively, because sICAM-1 one of the major adhesion molecules that inhibits natural killer cell-mediated cytotoxicity, resulting in defective immune surveillance, it is involved in the implantation and development of endometriotic lesions. To date, studies have shown an increase or decrease, of sICAM-1 levels (plasma/serum) in women with endometriosis compared with controls. In addition, no significant differences of sICAM-1 levels have been reported. This discrepancy may be due to different study designs, ELISA kits, or types of blood specimens, or to varying phases of the menstrual cycle [10].

Barrier and Sharpe-Timms in 2002 showed significant aberrations in levels ofICAM-1 in women with stage III and IV of endometriosis. These findings might shed light on the pathogenesis of endometriosis and be useful in the development of biochemical markers for disease stage [11].

Significant aberrations in levels of circulating adhesion molecules were found in women with stage III and IV endometriosis. These findings might shed light on the pathogenesis of endometriosis and be useful in the development of biochemical markers for disease stage [12]. Although the present study tends to support a role of sICAM-1 in the

development of endometriosis, serum concentrations of this molecule do not seem to be an effective indicator for the diagnosis of either the early or advanced stage of endometriosis. Other researchers give an idea about a panel of five biomarkers (CA-125, vascular endothelial growth factor (VEGF), Annexin V, Gd, and sICAM-1)), May et al. in 2010 [13] showed a sensitivity of 74-94% and a specificity of 55-75%. These results should be prospectively evaluated. Steff et al. in 2004 [14] have another opinions, serum levels of sICAM-1 during the luteal phase of the cycle are not able to discriminate women suffering from endometriosis from control. When confounders are taken into account, these results underline the importance of careful identification of confounders, based on patients' demographic and clinical data in studies aiming at discovering diagnostic markers for endometriosis.

Increased plasma Gd A levels have been observed in patients with endometriosis in this case-control study the sensitivity and specificity of the test by this marker are 76%, 85% respectively. Gd A with known angiogenic, immunosuppressive, and contraceptive effects, could contribute to the development of endometriosis and endometriosis-related infertility [7], this result is incompatible to others like Vdolazkaia et al. 2012 who have been conducted a study to assess the level of GdA, which found in high serum level in women with endometriosis (n=57) in comparison with control group undergoing sterilization or having ovarian cyst (n=42) the sensitivity of this test was 82.1% and specificity 78.4% that proposed the possible use of Gd A with panel biomarker for the diagnosis ultrasound-negative endometriosis[15].

Regarding Gd A, other study was adapted this, which proved that adolescent girls with endometriosis had significantly higher peritoneal fluid levels of IL-6, TNF-α and Gd A. Peritoneal IL-6, TNF-α and Gd A provided a good method of discrimination between subjects with endometriosis and controls. Using cut-off points for peritoneal fluid IL-6 (90.0 pg/mL), TNF-α (3.0 pg/mL) and Gd A (60.0 ng/mL), with high odds ratios (10.2; 14.6; 2.2) were obtained in the prediction of endometriosis in adolescents [16].

Moreover, Gd A is not only produced in the glandular epithelium of secretory endometrium, but also is shed from endometriotic lesions into the peritoneal fluid and serum. Increased plasma glycodelin levels have been observed in patients with endometriosis [17].

In conclusion, Gd A and sICAM-1in serum may be useful as noninvasive test for diagnosis of endometriosis in all stages. Further study with large sample size can be recommended to confirm this result.

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Authors Contribution:

Daraj: Data collection and drafting of the article. Dr. Abbas: Design of the work, data interpretation, drafting and critical revision of the article. Dr. Allaa: samples collection.

Conflict of interest

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Correspondence to Hadeel A. Draj
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Detection of Parvovirus B19 in Bad Obstetric History by Using Real Time PCR

Lina F. Abdulhassan[1] *MSc,* **Huda D. Hathal**[1] *PhD*, **Thuraya H. Abdullah**[2] *CABOG, FICOG*

[1]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Dept. of Gynecology and Obestetric, Al- Imamein Al-Kadhimein Medical City, Baghdad, Iraq

Abstract

Background Human *parvovirus* B19 (B19V) is a small single-stranded DNA virus. Infection during pregnancy can cause a variety of signs of fetal damage. The risk of adverse fetal outcome is increased if maternal infection occurs during the first two trimesters of pregnancy but may also happen during the third trimester.

Objective to determine the screen of *parvovirus* B19 in pregnant women with bad obstetric history by real time polymerase chain reaction (PCR).

Methods Two hundred Plasma and 200 placental tissue samples were collected from all pregnant women enrolled in this study. Three ml whole blood was collected in sterile EDTA-blood tube. Plasma was obtained by centrifugation of whole blood. Twenty-five grams of the placental tissue was homogenized with 10 ml of PBS by using tissue homogenizer for about 1 min at 4 °C.

Results 40 (20%) out of 200 plasma samples were real time PCR positive, the remainder 160 (80%) were real time PCR negative. Nineteen (9.5%) out of 200 placental tissue samples were positive for B19 real time PCR the remainder 181 (90.5 %) were real time PCR negative. All placental tissue positive (n=19) were positive by real- time PCR in plasma samples (n=40). Out of 40 pregnant women presented with positive parvoviruses results in current study demonstrated 21 (52.5%) gave abortion in first trimester and only 8 (20%) gave abortion in second trimester.

Conclusion *Parvovirus* B19 is common and highly distributed among pregnant ladies in this study and there is a significant association between B19 positivity and adverse pregnancy outcome.

Keywords *Parvovirus* B19, bad obstetric history, adverse pregnancy outcome, non-immune hydrops fetalis

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List of abbreviations: ANOVA = One-way analysis of variance, BOH = Bad obstetric history, CMV = Cytomegalovirus, EI = Erythema infectiosum, HB19V = Human *parvovirus* B19, NIHF = Non-immune hydrops fetalis.

Introduction

Human *parvovirus B19* (HB19V) is a small single-stranded DNA virus. Human *parvovirus B19* is the causative agent of erythema infectiosum (EI), a disease common in children. Studies have shown that intrauterine HB19V infection is related to non-immune hydrops fetalis (NIHF) and stillbirth [1, 2].

Approximately 1-3% of susceptible pregnant women without a known history of exposure to HB19V will seroconvert during pregnancy. Up to 50% of susceptible pregnant women exposed to HB19V through household contacts will develop seropositivity, while the rate of seroconversion is 20-30% when the exposure occurs in day care centers or school [3]. The risk of transmission to the fetus has been reported in the range of 17-33% but infection remains

asymptomatic in most cases. However, a wide range of adverse outcomes have been reported in association with intrauterine HB19V infection including: spontaneous abortions (rate of 14.8% if infected prior to 20 weeks gestation and 2.3% if the infection occurs after 20 weeks gestation); occurrence NIHF; congenital anomalies including central nervous system, craniofacial and eye anomalies [4]. Infection during pregnancy can cause a variety of other signs of fetal damage. The risk of adverse fetal outcome is increased if maternal infection occurs during the first two trimesters of pregnancy but may also happen during the third trimester [5].

HB19V can causes severe fetal anemia as a result of fetal erythroid progenitor cells infection with shortened half-life of erythrocytes, causing high output cardiac failure and therefore NIHF [5].

This study was carried out to determine the associated of HB19V in pregnant women with bad obstetric history.

Methods

Blood and placental tissue samples were collected from 200 pregnant women with bad obstetric history, attending the Gynecology outpatient clinics, wards and emergency unit in Al-Imamein Al-Kadhimein Medical City, and Baghdad Teaching Hospital during the period from December 2015 to May 2016. Three ml whole blood was collected in EDTA-blood tubes and plasma was obtained by centrifugation of EDTA-blood tube at 5,000 rpm for 5 min. While placental tissue twenty-five grams was homogenized with 10 ml of phosphate buffer solution by using tissue homogenizer for about 1 min at 4°C [6]. The resulting suspension was subjected to two freeze-thaw cycles to further break the cell membranes. After that, the homogenate centrifuged for about 15 min at a speed of 5000 rpm and temperature of (2-8) °C. The supernatant then collected carefully, both samples were stored at (-20°C) till DNA extraction.

Exclusion Criteria

Patient with a diabetic through HbA1c level, anti-phospholipid syndrome through anti-cardiolipin test and Rh, ABO incompatibility were excluded from this study.

Molecular method for diagnosis of Parvovirus B19

DNA was extracted from placental tissue and plasma using DNA isolation kit ((DNA-sorb-B (Sacace)/Italy) Kit) according to the manufacturer's instruction. The concentration and purity of the purified DNA was quantified by the use of nanodrop instrument following the instruction of the manufacturer, RT-PCR TaqMan assay using real time amplification with fluorescent reporter dye probes specific for HB19V and Internal Control (IC) were used in current study.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) was used for all statistical analysis; Chi-square used for categorical variables (Fishers exact test was used when expected variables were less than 5) and t-test was used to compare between two means. One-way ANOVA analysis was used to compare between more than two means. A two-sided significant level of 0.05 was considered to indicate a statistically significant difference.

Results

Molecular diagnostic method for detection of Parvovirus B 19 by real-time PCR

Results improved that 40 (20%) out of 200 plasma samples were real-time PCR positive, the remainder 160 (80%) were real-time PCR negative. Nineteen (9.5%) out of 200 placental tissue samples were positive for HB19V real-time PCR the remainder 181 (90.5 %) were real- time PCR negative.

All placental tissue positive (n= 19) were positive by real-time PCR in plasma samples (n=40). Real-time PCR was performed in duplicate for each sample that gave positive results.

The prevalence of positive HB19V in relation to age groups is presented in Table (1) and table

(2). In current study, 40 pregnant women proved as HB19V positive in plasma and 19 in placental tissue out of 40, the highest prevalence was observed in age group (30-39) years in both plasma 17 (28.3 %) and placental tissue 7 (11.7%). The association between age and positive pregnant women to parvoviruses was statistically significant in plasma (P=0.002) and highly significant in placental tissue (P<0.001).

Table 1. Correlation between B19 positive plasma cases and age of the pregnant women

Age	Positive viremia		Negative viremia		χ^2	P
	No.	%	No.	%		
<20 years	2	10.5	17	89.5	14.6	**0.002 Significant**
20-29 years	15	13.6	95	86.4		
30-39 years	17	28.3	43	71.7		
≥40 years	6	54.5	5	45.5		

Table 2. Correlation between B19 positive tissue cases and age of the pregnant women

Age	Positive viremia		Negative viremia		χ^2	P
	No.	%	No.	%		
<20 years	1	5.3	18	94.7	29.8	**<0.001 Highly Significant**
20-29 years	5	4.5	105	95.5		
30-39 years	7	11.7	53	88.3		
≥40 years	6	54.5	5	45.5		

Parvoviruses B19 in plasma and tissue with pregnancy number and pregnancy outcome

There was highly statistical significant between HB19V positive in plasma in associated with pregnancy number (P<0.001) and highly significant association (P<0.001) with pregnancy outcome table (3).

Table 3. Correlation between *Parvovirus* B19 in plasma and gravidity

Variable		Positive plasma		Negative plasma		χ^2	P
		No.	%	No.	%		
Pregnancies number	1-2	7	10.0	63	90.0	17.2	**<0.001 Highly Significant**
	3-4	12	15.8	64	84.2		
	>4	21	38.9	33	61.1		
Pregnancies outcome	Normal	2	2.7	73	97.3	22.5	**<0.001 Highly Significant**
	Adverse	38	30.4	87	69.6		

The association between HB19V positive in placental tissue compared to the number of pregnancy and pregnancy outcome were presented in table (4), there was a statistical significant association (P<0.006) between increased pregnancies number compared to positive parvoviruses and highly significant differences (P<0.001) with pregnancy outcome.

Table 4. *Parvoviruses B19* in placental tissue compared to pregnancy number and pregnancy outcome

Variable		Positive tissue No.	Positive tissue %	Negative tissue No.	Negative tissue %	χ^2	P
Pregnancies number	1-2	3	4.3	67	95.7	10.3	**0.006 Significant**
	3-4	5	6.6	71	93.4		
	>4	11	20.4	43	79.6		
Pregnancies outcome	Normal	0	0.0	75	100	12.5	**<0.001 Highly Significant**
	Adverse	19	15.2	106	84.8		

Type of abnormality of parvoviruses B19 in compared to gestational age and abortion

Out of 40 pregnant women presented with positive parvoviruses results in current study demonstrated 21 (52.5%) gave abortion in first trimester and only 8 (20%) gave abortion in second trimester there was highly statistical differences between gestational age and abortion in positive cases (P<0.005) (Table 5).

Table 5. Distribution of positive cases with gestational age and abortion in current pregnancy

Parameter		Positive tissue No.	Positive tissue %	Negative tissue No.	Negative tissue %	χ^2	P
Pregnancies number	1-2	3	4.3	67	95.7	10.3	**0.006 Significant**
Pregnancies outcome	Normal	0	0.0	75	100	12.5	**<0.001 Highly Significant**

A highly significant association was observed between women with high B19 viral load in plasma and in placental tissue and adverse pregnancy outcome (p<0.001) (Table 6).

Table 6. The association between abnormal pregnancy outcome and viral load

Variable		Normal No.	Normal %	Adverse No.	Adverse %	χ^2	P
Viral load in plasma	**Positive (40 cases)**	2	5.0	38	95.0	22.5	**<0.001 Highly Significant**
	Negative (160 cases)	73	45.6	87	54.4		
Viral load in tissue	**Positive (19 cases)**	0	0.0	19	100	12.5	**<0.001 Highly Significant**
	Negative (181 cases)	75	41.4	106	58.6		

Fishers exact test

Adverse pregnancy outcome and B19 viremia

Analysis of patients that were subsequently proved to be real-time PCR positive for HB19V in association with adverse pregnancy outcome in plasma and placental tissue was studied and the results was clarified a highly significant association as shown in table (7) with P<0.05 (P=0.0001).

Table 7. The relation between adverse Pregnancy outcome and B19 viremia

Type of adverse pregnancy outcome	Positive plasma		Positive tissue	
	No.	%	No.	%
Abortion	21	52.50	8	42.1
Still birth	13	13.00	6	31.5
Congenital abnormalities	3	7.50	3	15.7
Total	40	100%	19	100%
Chi-square	11.367		9.0294	
P-value	0.0001**		0.00216**	

** (Highly significant).

Discussion

Relation of demographic data in study group

The present study found highly significant association in studied group with adverse pregnancy outcome with different types such as stillbirth, abortion and congenital abnormality, adverse pregnancy outcome is any event which reduces the chance of having a healthy baby.

The mechanism of how some pregnancies lead to adverse maternal outcome is not fully understood though endothelial dysfunction [7], the findings of present study supported evidence are available in the study that reported that advanced maternal age and gestational hypertension has been suggested to play a role in adverse pregnancy outcome [7,8].

Relationship between maternal age and infection with Parvovirus B19

In this study, results showed increased age of women was associated significantly with positive infection with HB19V infection in plasma and placental tissue. This finding is consistent with results of Quemelo et al. [9], which showed that HB19V infection increases parallel with increased age of women.

Some postulate refer that older people may succumb to viral infection as a result to exaggerated immune responses, these age-elevated IL-17 responses induce a lethal immune pathology during viral infection. These responses synergize with defective Parvovirus B19 clearance with aging noted by impaired IFN-α responses [10].

Correlation of Parvoviruses B19 in plasma and tissue with pregnancy number and pregnancy outcome

Current study showed that women with increased number of multipara (>4) were significantly associated with HB19V infection of plasma and placental tissue. This is similar to results of Adam et al. [11]. It is known that infection occurs throughout adult life increasing the seroprevalence rate from ~60% at age 18 years to >80% in geriatric populations (10). Moreover, multigravida B19 virus-susceptible women are more liable to acquire HB19V infection from their living children [12].

In Valeur-Jensen study; they found that the seroprevalence of HB19V in mothers significantly increased with the number of their living children, particularly, children aged 5-7 years [13].

The higher risk of infection has to be due to a higher exposure rate among women with children at home. This fact most likely represents a mixture of a higher exposure rate,

as multigravida women generally have a higher rate of daily contacts compared with nulliparous women, and probably a reduced level of active immunity, because of the stress factor. Furthermore, serious medical disease may be found in multigravida women which causes clearly impairs the level of active immunity and thereby increases the susceptibility to infection [14].

Present study revealed a highly significant association between infected women with HB19V in plasma and abnormal pregnancy outcome ($p<0.001$). This finding is consistent with results of Kishore et al. [15], which reported that women with bad obstetric history (BOH) and/or pregnancy complications had a high frequency of TORCH and HB19V infections causing fetal wastage, intrauterine growth restriction, nonimmune hydrops fetalis and congenital malformations.

This study found a highly significant association between women with HB19V placental tissue infection and abnormal pregnancy outcome ($p<0.001$). This finding coincides with results of Lamont et al. [16], which revealed that if pregnant women develop HB19V infection, there is a 30% chance of fetal transmission, which is associated with adverse fetal outcomes.

Relationship between gestational age and Parvovirus B19 infection

The common abortion cases in present study including women infected with Parvovirus B19 infection were in first trimester. This is similar to results of Kishore et al. [15]. The risk of fetal complications depends largely upon the gestational age at the time of maternal infection with HB19V. It seems that the highest risk for fetal loss if maternal infection occurs during weeks 9-16 of pregnancy, then infection is reduced in the second half of pregnancy and rare if infection occurs in the last 2 months [17].

The possible explanation for these results because transplacental transmission is more likely to occur due to the presence of the P-antigen, which is a glycolipid (globoside) present in the trophoblast. This receptor is highly expressed in the first and second trimester, but virtually non-existent in the third trimester [18]. This period showed growing of fetus organ and hemopoiesis occurs in the fetal liver and due to the increased demand from the growing fetus, there is a 34-fold increase in red blood cell mass, These unique circumstances make the fetus especially vulnerable to any insult with respect to erythropoiesis [19].

Relationship between adverse Pregnancy outcome and B19 viremia

The current data showed highly significant association among infected women with Parvovirus B19 with different type of adverse pregnancy outcome. This finding is consistent with many studies done by Leduc et al. [20] and Watt et al. [21] who found that, the adverse pregnancy outcome of HB19V infection including abortion, still birth and congenital abnormalities increased in children of mothers with parvovirus infection in pregnancy.

Relationship between adverse pregnancy outcome and viral infection were analyzed by large group of authors who suggest that the type of response initiated in the placenta may determine the immunological response of the mother and consequently, the pregnancy outcome [22,23]. It is well accepted that in viral infection during pregnancy will lead to embryonic and fetal death, induce miscarriage or induce major congenital anomalies. However, even in the absence of placental transmission, the fetus could be adversely affected by the maternal response to the infection [24].

Viral infection of the placenta lead to stimulate high circulating levels of inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-α, which causes fetal inflammatory response syndrome (FIRS), even though the virus is not able to reach the fetus, which lead to adverse pregnancy outcome [25].

In conclusion, HB19V is common and highly distributed among pregnant ladies in this study. There is a significant association between HB19V positivity and adverse pregnancy outcome. The rate of abortion in positive HB19V is the most adverse pregnancy outcome followed by stillbirth.

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Authors Contribution
Abdulhassan: DNA extraction, molecular, real time PCR methods diagnosis, analysis and interpretation of result and statistical analysis. Dr. Hathal: drafting the article and revising it critically for important intellectual content. Dr. Abdullah: samples and patients selections.

Conflict of interest
The authors declare no conflict of interest.

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Correspondence to Lina F. Abdulhassan
E-mail: linaabdalhassan@gmail.com
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Detection of ExoT Gene in Local Isolates of *Pseudomonas auroginosa* in a Sample of Burn Infection

Rana A. Hanoon[1] *PhD,* **Ibtisam G. Auda**[2] *PhD*, **Ismail H. Aziz**[3] *PhD*

[1]Scientific Research Center, College of Science, University of Duhok, Iraq, [2]Dept. of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq, Institute of Genetic Engineering and Biotechnology, University of Baghdad, Iraq

Abstract

Background *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic microorganism that requires damaged mucus membranes and epithelial tissues to cause acute infections. It had been stated that *P. aeruginosa* alters mammalian cytokinesis in a type III secretion system and exotoxin T (ExoT)-related way.

Objective To identify exoT gene local isolates of *P. aeruginosa* isolated from burn infections.

Methods Forty bacterial isolates of *P. aeruginosa* (isolated from burn infection) were identified by standard laboratory methods and polymerase chain reaction (PCR) technique was applied for the detection of the gene encoding for ExoT.

Results The results showed that PCR amplification of exo T gene occurred in 24 (60 %) isolates out of the enrolled 40 isolates of *P. aeruginosa* while 16 (40 %) of the isolates showed negative amplification reactions.

Conclusion It appeared that exoT can be a significant virulence factor expressed by 60 % of *P. aeruginosa* isolates as indicated by positive PCR-amplification results.

Keywords Burn infections, Exotoxin T, type III secretion system, PCR, *P. aeruginosa*

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List of abbreviations: PCR = Polymerase chain reaction, *P. aeruginosa = Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic bacteria that has the ability to infect virtually all kinds of tissues, affect immunosuppressed patients and can cause nosocomial infections [(1)]. Burn affected individuals, patients with assisted ventilation, and cystic fibrosis (CF) victims are specifically susceptible to be infected with *P. aeruginosa*. It has been documented that these bacteria are the leading cause of increased morbidity and mortality in persons with cystic fibrosis [(2)]. *P. aeruginosa* is responsible for an elevating percentage of infections acquired in the modern hospitals, especially in intensive care units (ICU) and in patients with urological disorders and has held almost unchanged position in the rank order of pathogens causing ICU infections during the last 4 decades [(3)].

P. aeruginosa utilizes a wide variety of virulence factors, which either help the pathogen to adhere to target cells or act as toxins. These toxins are either released by passive transport from the cells or actively secreted via one of the three secretion systems

namely, type I secretion system (T1SS), type II secretion system (T2SS) or the type III secretion system (T3SS) [4].

The complex III secretion system is an essential and newly recognized virulence factor of *P. aeruginosa* responsible for injecting certain toxin molecules into the target mammalian cells. The chromosome of *P. aeruginosa* harbors the genes encoding for type III secretion system in an evenly distributed manner [5].

P. aeruginosa has been shown to have the so-called type III secretion system (T3SS), along with a group of effector molecules (ExoT, ExoS, ExoY and ExoU). These protein factors can be directly delivered to the host cells; and once transferred, can elicit various host responses, facilitating successful dissemination and infections [6].

ExoT and ExoS, the first enzymes in this group are known. ExoT produced during the release and escape of pathogens. In addition, it has ADP-ribosil transferase activity, which acts similar pathology cholera toxin [7]. ExoT attacks host kinases mainly responsible for focal adhesion and eventual phagocytosis and has been linked with dissemination of infection from the lung to the liver in mice model and induction of apoptosis in HeLa cells [8].

The present study aimed to identify exoT gene local isolates of *P. aeruginosa* isolated from burn infections.

Methods

Sampling

Burn wound swabs were obtained from forty patients. Specimen collection started from January to April 2012 from the laboratories of Al-Imamein Al-Kadhimein Medical City, Baghdad. Identification of the obtained isolates was performed according to previous work [9].

DNA Extraction

Wizard genomic DNA purification kits (Promega®, USA) was used for the extraction of bacterial DNA as indicated by the manufacturer's instructions. Agrose gel (1%) electrophoresis on 1 % agarose was applied to confirm the results of DNA extraction [10].

Primers

Primers were synthesized by Bioneer ® (South Korea). Polymerase chain reaction (PCR) product size and melting temperature were 1000 bp and 45 °C, respectively (Table 1).

Table 1. Primers sequences and molecular weight of the relevant PCR products of exoT

Gene	Forward primer	Reverse primer	Product size (bp)
*exo*T	5′TCACTGCAGTTCCGCGTGCTCCGACG 3′	5′TCAGGTACCTGCTGGTACTCGCCGTT -3′	1000

Polymerase chain reaction (PCR)

Polymerase chain reaction was performed by adding 3 µl of the bacterial DNA to the preloaded master mix eppendorff tubes (AccuPower PCR premix-® (South Korea)) then 2.5 µl (10 pmol\µl) of the specific primers was also added, the final volume of 20 µl was attained by adding distilled water. Table 2 describes the running conditions for the amplification of exoT (Table 2).

Note: Running conditions were adopted after several trials depending upon other's work [11].

Agarose Gel Electrophoresis

Agarose at 1 gm, 1.5 gm was dissolved in 100 ml of 1X tris-borate EDTA buffer for genomic DNA and PCR products, respectively. These mixtures were then solubilized by heating, then they were left to cool at 40°C and poured into the taped plate [10].

Table 2. Cycling conditions for the PCR-amplification of exoT gene of *P. aeruginosa*

Step	Temperature (°C)	Time (minutes)	No. of Cycles
Initial denaturation	94	3	1
First loop:			
Denaturation	94	30 seconds	
Annealing	50	30 seconds	40
Extension	72	1	
Final extension	72	5	

Results

The results of the present work revealed that all the enrolled bacterial isolates were primarily identified as *P. aeruginosa* because they looked as gram negative, oxidase positive rods, and able to grow at 42 °C, the growth of the colonies characterized by sweet musty odor. Oxidation/fermentation test was also applied for the confirmation of the identity of the isolates.

Figure 1 shows the genomic DNA of the bacterial species enrolled in the current work.

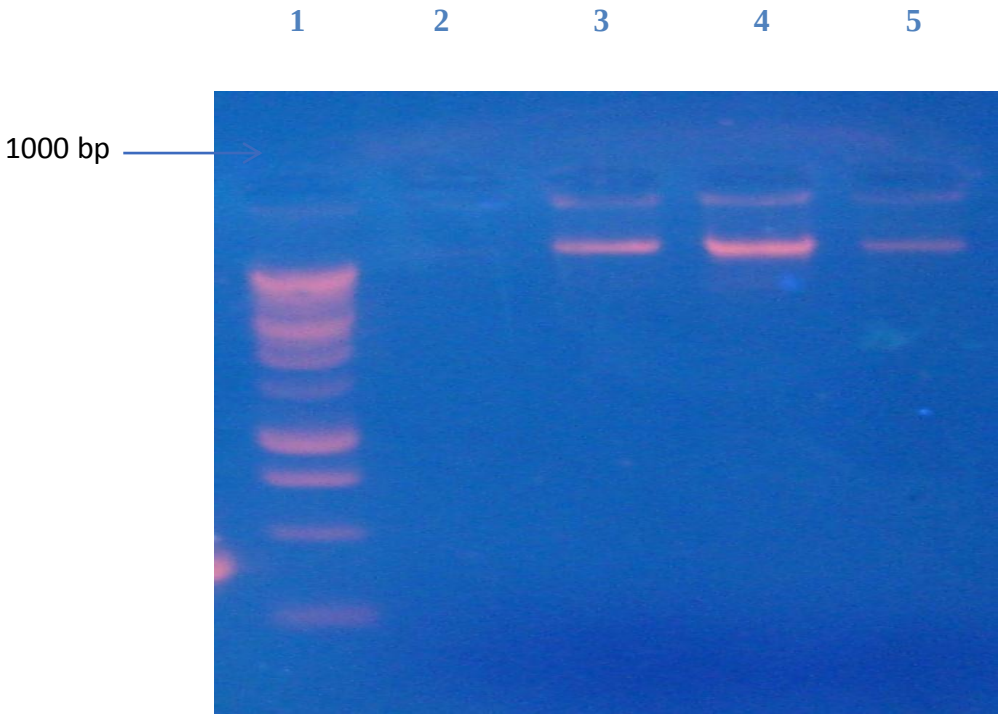

Figure 1. Bacterial chromosomal DNA. Lane 1: 1000 bp molecular marker, lane 3, 4, and 5. Bands run on 1% agarose at 4 V/cm for 60 minutes

PCR experiment indicated that exoT gene was successfully amplified in twenty-four (60 %) out of the forty isolates of *P. aeruginosa*, while only sixteen (40 %) isolates were shown to be negative (Figure 2).

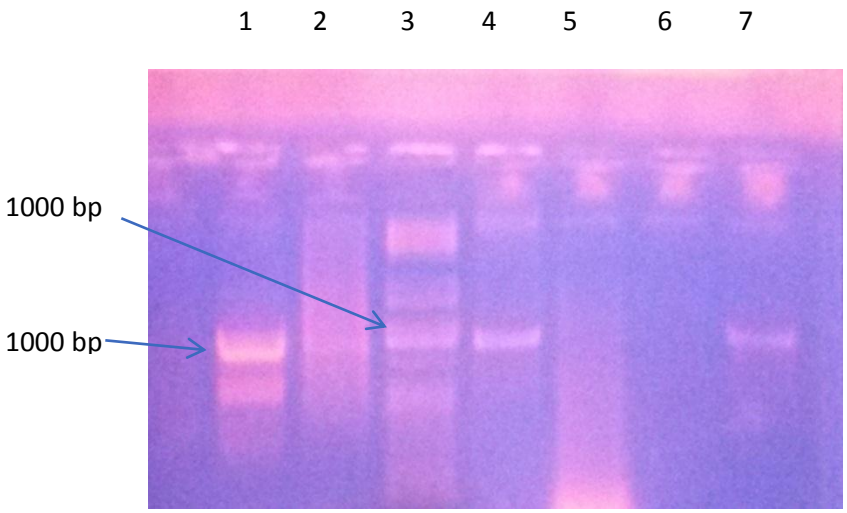

Figure 2. Electrophoresis of PCR products of exoT of *P. aeruginosa*. From left to right: Lane 3: 1000 bp ladder, lane: 1, 2, 4 and 7 amplification products of exoT gene (1000 bp) Lane 5 and 6 negative results. Electrophoresis conditions were: 5V/cm for one hour, the concentration of agarose gel was 1.3%

Discussion

The type III secretion system (T3SS) is a needle-like nanomachine that delivers virulence proteins (exotoxins) directly into target cells to initiate infection. These exotoxins trigger and maintain infection by altering target cell functions, such as signaling pathways, secretory trafficking, constant movement of the cytoskeleton, and cellular reaction to inflammatory stimuli. T3SS is fundamental for survival and pathogenesis of several Gram-negative organisms including *Pseudomonas, Salmonella, Escherichia, Shigella, Yersinia, and Chlamydia spp*. [12]. This complex cellular machinery acts in a very well-organized manner and can modify the target cell in many variable ways. The opportunistic nature of *P. aeruginosa* in humans is well defined, therefore, it is unlikely that type III secretion system has evolved as the result of survival pressure within host cells. The usual target of the *P. aeruginosa* T3SS is unknown. Probably, this system may have been established to combat nearby predators (e.g. amoebae that inhabit the soil and water), and broad conservation of targeted materials across eukaryotic creatures culminating in a system that is effective against human cells as well [13].

Effector proteins delivered by type III secretion system have been shown to have important contribution to the virulence of *P. aeruginosa* in a variety of in vivo animal studies [14-16]. Moreover, analysis of *P. aeruginosa* clinical isolates has also revealed a prominent correlation between production of T3SS effectors and enhanced severity of the disease with an elevated mortality rate [17]. Interestingly, the significance of T3SS in dictating clinical consequences and enhancing pathogenic process in animal studies is well documented, nevertheless, clinical isolates usually do not express T3SS in vitro and can cause illness in a T3SS-independent path [18].

The results of the current work contradicted those of other study which stated that exoT gene was found in 100 % of the enrolled *P. aeruginosa* isolates. The difference between the results might be attributed to the variation of the samples, from which the isolates were obtained and that environmental isolates of P. aeruginosa may show varying expression patterns for the virulence factors [19].

In s study conducted in Iran, over 144 clinical and environmental isolates of *P. aeruginosa*, it was shown that 37.9% of the isolates were positive for the production exoT when clinical

samples were considered while much less figure of 27.8 % was obtained when hospital settings isolates was estimated [20]; both results showed less frequent isolation of *P. aeruginosa* when compared to the result of the current study.

It was postulated that the ExoT gene is not a variable trait since it has been found in all the examined isolates [21,22] and that the existence of this gene in all the studied environmental isolates indicates that there may be selective advantage for this gene in hospital environments [8].

In a separate study, exoT gene prevailed in case of wound samples (95 %); despite higher figure that what is recorded in the present work, both studies support the previous knowledge indicating an important role for ExoT in bacterial dissemination [23]. It is of note to state that the abundance of type III secretion genes in clinical isolates is in line with its critical role in the pathogenicity of *P. aeruginosa* and the understanding of the particular contribution of ExoT to the clinical outcome of the infection may have substantial guiding for the therapeutic approach of patients infected with *P. aeruginosa* [24].

In conclusion, exoT gene plays an important role in the infectious process caused by *P. aeruginosa*.

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Authors Contribution:

Dr. Auda conducted the sampling, isolation, and molecular work. Rana and Dr. Aziz guided and finished writing and editing the study.

Conflict of interest

The authors declare no conflict of interest.

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Correspondence to Rana A. Hanoon
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Prevalence and Diagnosis of sexually Transmitted Pathogens in A Sample of Iraqi Women: A Molecular Study

Mariam K. Ali[1] *MSc,* **Huda D. Hathal**[2] *PhD*, **Hala A. Almoayed**[3] *ABOG*

[1]Dept. of Microbiology, College of Medicnie, University of Baghdad, Iraq, [2]Dept. of Microbiology, [3]Dept. of Gynecology and Obstetrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Sexually transmitted infections (STI), also referred to as sexually transmitted diseases (STD) and venereal diseases (VD), are infections that are commonly spread by sex, especially vaginal intercourse, anal sex and oral sex. Most STIs initially do not cause symptoms. This results in a greater risk of passing the disease on to others. Symptoms and signs of disease may include: vaginal discharge, penile discharge, ulcers on or around the genitals, and pelvic pain.

Objective To detect the two microorganisms (*Gardnerella vaginitis (G. vaginalis)* and *Trichomonas vaginalis (T. vaginalis)*) in the same sample taken from women with genital tract infection by microbiological and molecular methods and to investigate the contributions of some socioeconomic factors and clinical features.

Methods Two hundred samples were collected from females attending the Gynecology out-patient department in the Al-Imamein Al-Kadhimein Medical City and Baghdad Teaching Hospital during the period from May 2014 to April 2015. Based on availability of full clinical information about each patient, high vaginal swabs were taken from females at different ages (15-54 years) representing patients group complaining of abnormal vaginal discharge with or without other symptoms, questionnaire was applied. The two diseases associated with vaginal infection include *G. vaginalis and T. vaginalis.* Each of the vaginal swabs collected was examined microscopically, whilst the remaining was preserved at -20 °C for DNA extracts were analyzed with the real-time poly meres chain reaction (RT-PCR).

Results In RT-PCR, the rate of infection was 120 (60%) *G. vaginalis*, and 34 (17%) *T. vaginalis*. Highest rate of infection in women with *G. vaginalis* was among age group (15-24) years and (25-34) years 38.3%, 35.0% respectively, the lowest rate was among age group (45-54) years 8.3 %. In *T. vaginalis*, the highest rate of infection was among age group (15-24) years 61.7 %, the lowest was among age group (35-44) years 8.8 % and on infection in age 45-54years.

Conclusion The commonest genital tract infections among women were *G. vaginalis and T. vaginalis.* Molecular methods are considered the gold standard for diagnosis, given the excellent sensitivities and specificities in diagnosis. Presence of clinical symptoms helps and lab diagnosis of infection. Vaginal swab samples showed that most common co-infection is between *G. vaginalis* cases and *T. vaginalis*.

Keywords Sexually transmitted infections, *Gardnerella vaginalis*, *Trichomonas vaginalis*, molecular study

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List of abbreviations: *G. vaginalis* = *Gardnerella vaginalis*, PCR = Polymerase chain reaction, STPs = Sexually transmitted pathogens, *T. vaginalis* = *Trichmonas vaginalis*, VEC = Vaginal epithelial cell

Introduction

The normal vaginal ecosystem is a complex micro environment with important interrelationships among endogenous microflora and their metabolic products, estrogen status and pH [(1)]. Lactobacilli maintain the normal vaginal pH (3.8-4.2) by producing lactic acid, stabilizing the vaginal ecosystem and hydrogen peroxide, suppressing the growth of gram-negative and gram-positive facultative and obligate anaerobes. Vaginitis is the inflammation and infection of vagina commonly encountered in clinical medicine. Diverse spectrums of

pathogenic agents were observed in the vaginal micro flora. Of these, *G. vaginalis* and *T. vaginalis* are responsible for majority of vaginal infections in women of reproductive age [1]. Abnormal vaginal discharge, itching, burning sensation, irritation and discomfort are frequent complaints among patients attending obstetrics and gynecology clinics. However, a number of vaginal infections present with few or no symptoms [2].

Vaginal infections are associated with a significant risk of morbidity in women. If untreated they can lead to pelvic inflammatory disease (PID), which can cause long-term sequelae, such as tubal infertility, ectopic pregnancy, reproductive dysfunction. Cervical dysplasia, increased risk of postoperative infection

G. vaginalis is the most common vaginal infection of reproductive age women and is the most frequently cited cause of vaginal discharge and malodor. Vaginal discharge constitutes a considerable problem for many women causing discomfort, anxiety affecting women. Some vaginal discharges are normal and can vary with age, use of contraceptives and menstrual cycle [3].

The protozoa *T. vaginalis* is a sexually transmitted parasite causing vulvovaginitis characterized by intense frothy yellow-greenish vaginal discharges, irritation and pain in the vulva and dysuria [4,5].

Recent studies have shown that polymerase chain reaction (PCR) increases the rate of *G. vaginalis* and *T. vaginalis* detection in mucocutaneous swabs. Although earlier PCR techniques were laborious, expensive and prone to contamination, newly developed real time PCR assays are fully automated [6]. This is a valuable feature for the use of PCR in routine clinical practice.

The objectives of this study were (1) to study the prevalence of two pathogens (*G. vaginalis* and *T. vaginalis*) in women with lived partner age (15-54) years by microbiological and molecular methods and to investigate the contributions of some socioeconomic factors and clinical features, (2) to study the association between *G. vaginalis* and *T. vaginalis* by real time-PCR.

Methods

Vaginal swabs collection

High vaginal swabs were collected from two hundred samples, women patients (symptomatic and asymptomatic) within reproductive age who attending the Gynecology Outpatient Department in the Al-Imamein Al-Kadhimein Medical City and Baghdad Teaching Hospital during the period from May 2014 to April 2015. Based on availability of full clinical information about each patient, high vaginal swabs were taken from females at different ages from 15-54 years. representing patients group complaining of abnormal vaginal discharge with or without other symptoms.

This research under went to the terms of ethical considerations and in accordance with the form prepared for this purpose by the Committee of Ethical Standards in the Collage of Medicine / University of Al-Nahrain.

Full information history was taken directly from the patient and information was arranged in an informative clearly detailed formula sheet.

Isolation of microorganisms

A-*Macroscopic examination*:

vaginal swabs were observed in terms of consistency, color, odor, viscosity and presence of blood and mucus and testing for pH.

B-*Microscopic examination*:

Microscopic examination of the vaginal discharge wet smears, were conducted immediately after collection of specimens at the respective clinics. All further processing of specimens was done in the laboratory.

1-Wet-mount preparation:

A wet-mount preparation is obtained by diluting the vaginal discharge with one or two drops of normal saline solution and placing it on a slide with a cover slip. Slide is examined microscopically using low power and high power of several fields for motile trichomonads. Microscopic examination of wet-mount preparation also detects "Clue cell"

which are vaginal epithelial cells that are coated with the coccobacilli.

2-Gram stain:

Vaginal smears were prepared by transferring vaginal secretions to glass slides. The slides were air-dried, heat-fixed and Gram-stained. Smears were examined under oil immersion (XlOO objective) and quantitated for the presence of "clue" cells, Gram-variable bacilli, *G. vaginalis*-like organisms.

3-Vaginal WBCs counts:

Vaginal WBCs were quantified after visualization of a minimum of fine fields (range 5-15 based on whether there was a paucity of WBCs present) under light microscopy at X 400. Vaginal WBCs count were routinely categorized into either ≤ WBCs in all visualized fields (representing minimal or no inflammation) or ≥ 5 WBCs in at least one field visualized (considered elevated and more suggestive of significant inflammation).

4-Culture:

Specimen was culture immediately on the laboratory media by rolling the cotton swab on one side of the plate then streaked by standard streaking method. The inoculated plates were incubated as follows:

1-One blood ager plate, MacConkeys ager plate, these were incubated aerobically at 37 °C (24-48 hr) and anaerobically by using anaerobic Jar at 37 °C for (24-48 hr).

2-Modified protease peptone ager plate, this medium was shown to increase the recovery of vaginal bacteria, the plate inoculated sealed and anaerobically at 37 °C for (6-7) days.

5-Biochemical test: Oxidase test, catalase test.

6-Molecular study.

(Sacace ™ Biotecnologies) is a single iplex RT-PCR kit Reagents and contents of kit, for the direct and qualitative detection of *G. vaginalis* and *T. vaginalis*.

The principle, procedure, of (DNA extraction, real-time PCR, nano drop and agarose electrophoresis) preparation sample, and interpretation of the results were as same as those in *G. vaginalis* and *T. vaginalis* determination method.

Statistical analysis

The Statistical Analysis System- SAS, program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage in this study.

Results

Demographic characteristics of population:

Of the 200 women examined, the age of the study population ranged from 15-54 years, Women aged 15-24 years had significantly higher prevalence of infection 67 (33.5%) than other age classes (Table 1). The lowest percentage of women aged participating were in the age group 45-54 year 10 (5%) statistically significant association between age of subject and occurrence of vaginal infection.

The study revealed that women with primary education have the highest number and percentage 63 (31.5%), while low percentage of infection was seen in higher educational level 17 (8.5%). Based on socioeconomic status, the moderate socioeconomic status had the highest rate 77 (38.5%) followed by low status 62 (31%).

In rural residence the infection rate 66 (33%) was significantly higher than infection rate in urban 90 (45%).

In parity, the infection percentage was increased in multipara status had the highest rate 85 (42.5%) followed by unipara status 49 (24.5%), and finally the nullipara status 22 (11%).

Table 1. Variable factors of sexually transmitted infections according to patients with questionnaire in the present study

Variables		No. of test cases	Positive cases No (%)	X^2	P value
Age group (year)	G1: 15-24	85/200	67 (33.5%)	9.725	0.0001**
	G2: 25-34	60/200	53 (26.5%)		
	G3: 35-44	30/200	26 (13.0%)		
	G4: 45-54	25/200	10 (5.0%)		
Education levels	Illiterate	54/200	44 (220%)	8.136	0.0026**
	Primary	72/200	63 (31.5%)		
	Secondary	42/200	32 (16.0%)		
	Higher	32/200	17 (8.5%)		
Socioeconomic classes	Low	75/200	62 (31.0%)	9.022	0.0008**
	Moderate	90/200	77 (38.5%)		
	High	35/200	17 (8.5%)		
Location	Urban	115/200	90 (4.5%)	4.317	0.0474*
	Rural	85/200	66 (33.0%)		
Parity	Nullipara	40/200	22 (11.0%)	8.166	0.0064**
	Unipara	65/200	49 (24.5%)		
	Multipara	95/200	85 (42.5%)		

* (P<0.05), ** (P<0.01)

Clinical Features of sexually transmitted disease:

Presence of clinical symptoms helps in the diagnosis of infection. The symptoms experienced by 156/200 infected women participating in this study are summarized in table (2).

Women with malodor signs represent the highest percent of cases 47/200 (23.5%). Followed by profuse discharge and vulvar itching 45/200 (22.5%), 26/200 (13%) respectively while joint pain, dysuria and cervical abnormalities were represented signs 20/200 (7.5%), 15/200(7%), 12/200 (4.5%) respectively.

In this study, all vaginal swab samples were examined in the laboratory by microscopic examination (Wet mount preparation and Gram's stain) for detection of (clue cell, *T. vaginalis* trophozoites), 95/200 (47.5%) samples found to be positive for *G. vaginalis* by Gram stain and 25/200 (12.5%) were found to be positive *T. vaginalis* mount preparation in wet as shown in table (3), figures (1), (2) and (3).

This study showed that *G. vaginalis* was isolated from (50%) of infection cases using culture method.

The sexually transmitted pathogens identified from patient group by RT- PCR

The PCR amplification were performed using RT-PCR. The detection of appropriate channels was used as follow channel (FAM) for detection *G. vaginalis* and *T. vaginalis*, channel (CY3) for internal control of DNA (ICD). Used for no evidence of inhibition of the amplification in any of the samples, with the internal control of the RT-PCR samples as shown in table (4), Figures (4) and (5) respectively. In Table (4) show that from 200 samples, only 154/200 samples were diagnosis to be infected by RT-PCR. These assays detected 120 (60%) cases of G. *vaginalis*, 34 (17%) cases of *T. vaginalis*.

Table 2. Frequency of clinical aspects in patient with sexually transmitted in disease

Symptoms and signs	No. of cases	No. of effected cases	(200) %	(156) %	χ^2	P- value
Vulvar itching	45/200	26/45	13	16.66	10.435	0.0001**
Malodor	55/200	47/55	23.5	30.12		
Profuse discharge	53/200	45/53	22.5	28.84		
Joint pain	20/200	15/20	7.5	9.61		
Dysuria	15/200	14/15	7	8.97		
Cervical abnormalities	12/200	9/12	4.5	5.76		

Table 3. Detection of sexually transmitted pathogen (STP) in patients with vaginal infection by microscopic examination

Sexually transmitted pathogen	Positive cases	Infection rate %
G. vaginalis	95/200	47.5
T. vaginalis	25/200	12.5
Total	120	60

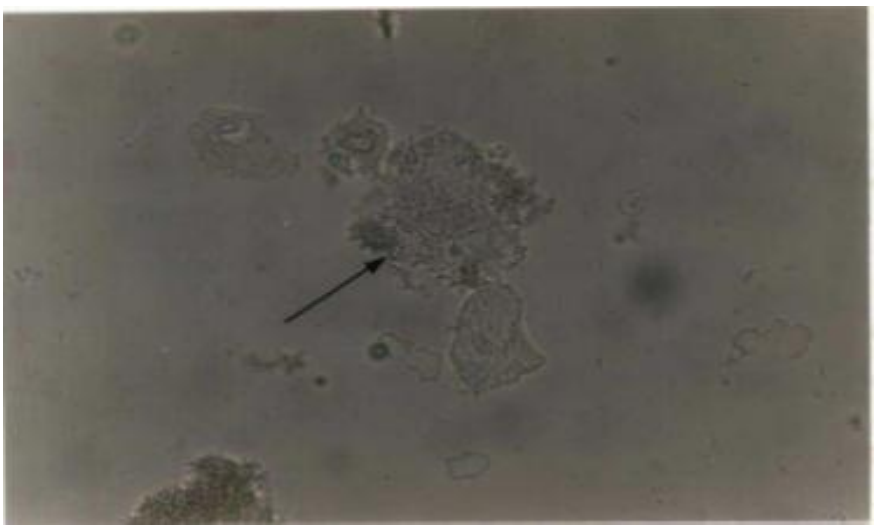

Figure 1. Clue cells as seen on vaginal wet smear microscopic preparation (40X objective)

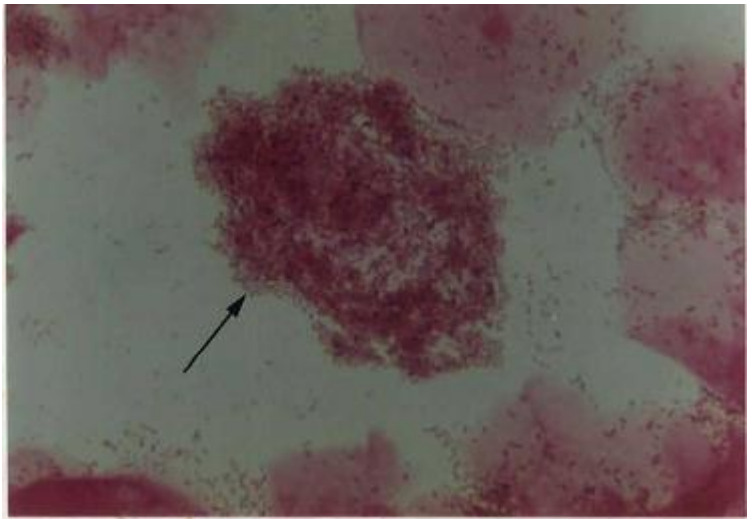

Figure 2. Clue cells as seen on A-vaginal wet smear microscopic preparation B- Gram stained smear with the presence of *G. vaginalis*

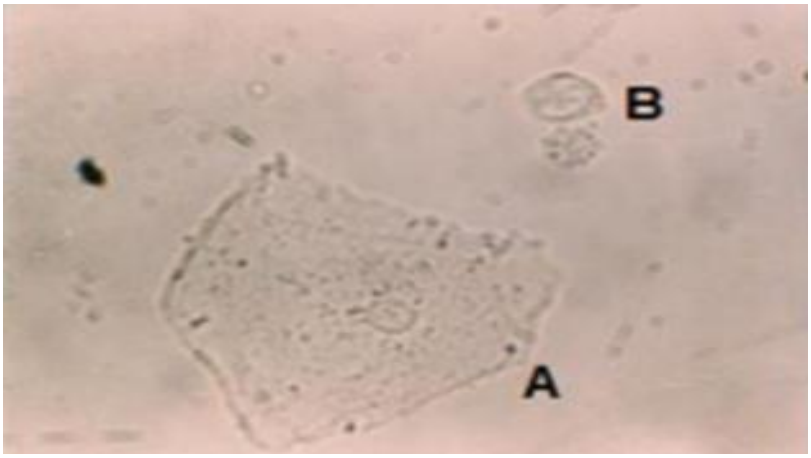

Figure 3. Wet mount preparation under light microscope 40X. A: epithelial cell B: two shapes of *T. vaginalis*

Table 4. The sexually transmitted infection identified from patient group according to type of pathogens by RT- PCR

Sexually transmitted pathogen	Multiplex RT- PCR	Infection rate %
G. vaginalis	120	60
T. vaginalis	34	17
Total	154	77

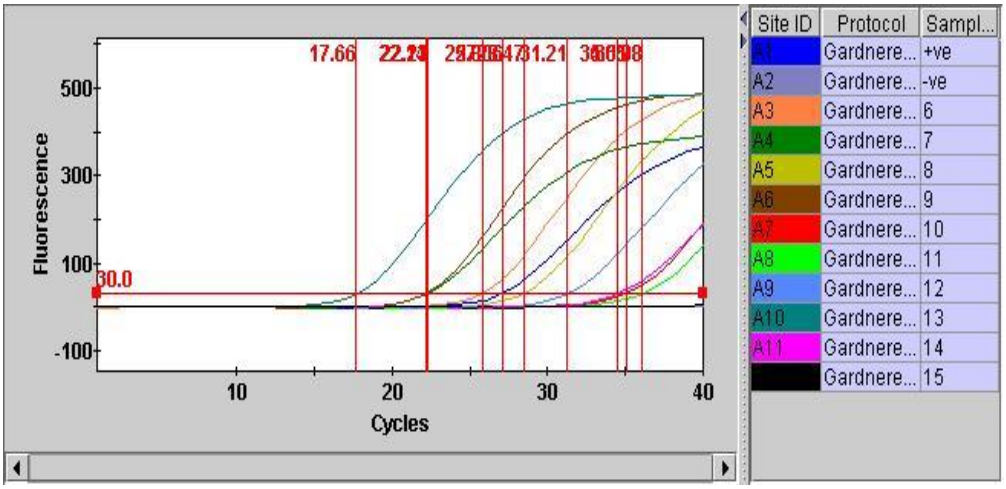

Figure 4. *G. vaginalis* RT-PCR results; positive sample (blue), negative sample (green), each one with internal control

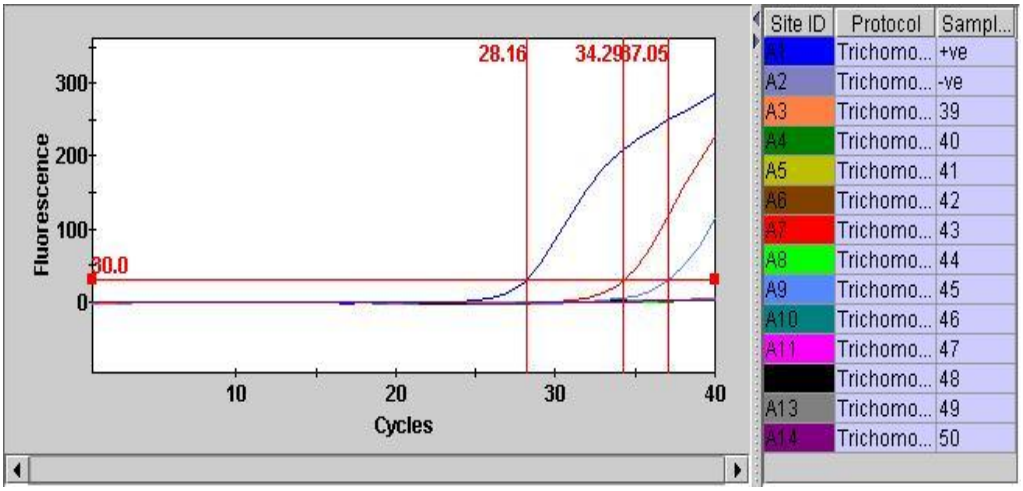

Figure 5. *T. vaginalis* RT-PCR results; positive sample (blue), negative sample (green), each one with internal control

Types of vaginal infection in relation to age group

The age groups that were subjected to this study ranged from 15-54 years. Table (5) showed highly significant relationship between age and *G. vaginalis* and *T. vaginalis* infection.

Highest rate of infection in women with *G. vaginalis* was among age group (15-24) years and (25-34) years 38.3%,35.0% respectively, the lowest rate was among age group (45-54) years 8.3 %.

In *T. vaginalis*, the highest rate of infection was among age group (15-24) years 61.7%, the lowest was among age group (35-44) years 8.8 % and on infection in age 45-54years.

Mix vaginal infection (co-vaginal infection)

Diagnosis of 120 *G. vaginalis* infections in vaginal swab samples and 34 cases for *T. vaginalis* by multiplex RT-PCR showed that Most common co-infection is between *G. vaginalis* cases and *T. vaginalis* were 30/156 (19.23%) cases of co-infection, in the present

study as is illustrated in Table (6). Statistically, there were highly significant differences among co-infection cases.

Table 5. Types of genital tract infection in relation to age groups

Sexually transmitted pathogen		Age groups / Year 15-24	25-34	35-44	45-54	Total	P value
G. vaginalis	No.	46	42	22	10	120	0.0001 **
	%	38.3	35.0	18.3	8.3	100	
T. vaginalis	No.	21	10	3	-	34	0.0001 **
	%	61.7	29.4	8.8	-	100	
Total	No.	67	52	25	10	154	
	%	43.5	33.8	16.23	6.5	100	

Table 6. Mixed of vaginal infection

Mixed infection	Positive cases	% from total	Negative cases	Total
G. vaginalis* + *T. vaginalis	30	19.48	124	154
Age groups	15-24	25-34	35-44	30
Positive cases	14	13	3	
Color of discharge	Yellow or green	White	Gray-White	30
Positive cases	13	17	-	
Consistency of discharge	Flocculent	Thin homogenous	Crude	30
Positive cases	-	30	-	

Discussion

Of the 200 women examined, the age of the study population ranged from 15-54 years, Women aged 15-24 years had significantly higher prevalence of infection 67 (33.5%) than other age classes. The lowest percentage of women aged participating were in the age group 45-54 year 10 (5%); there was statistically significant association between age of subject and occurrence of vaginal infection. This result agreement with Hassan et al. (2005) in Basrah [8], who showed that the highest percentage of infection occurred (47.2%) at the same age group and disagreement with other studies like Bahram et al. (2009) in Iran [9]. In Vietnam it was 8.7% (4/64) [10], while in Turkey, 10% (2/20) menopausal vaginal infection patients was recorded [11].

Women in the age groups (15-24), (25-34), (35-45) years had the highest prevalence of infection compared with older age category in a study of women according to the pH level, vaginal discharges were varied with age, use of contraceptives, menstrual cycle and with the estrogen level, douching used. Sexually transmitted pathogens (STPs) regard as the most serious causative agent for vaginal infection. However, while vaginal infection is

possible increased prevalent in reproductive ages due to biological factors such as age, hormonal changes, cervical ectopic [12-14].

The study revealed that women with primary education have the highest number and percentage 63 (87.50%), while low percentage of infection was seen in higher educational level 32 (31.5%). Based on family income, moderate socioeconomic status had the highest rate 77 (38.5%) followed by low status 62 (31%). In Iraq, previous studies in Baghdad and Al-Najaf showed that the uneducated women (illiterates) were more associated with the disease than other age classes [15,16]. The low infection rate was shown in women who have high educational level, and this agree with number of demographic studies, which showed that higher educational level was the lowest level associated with the disease [17].

In rural residence, the infection rate 66 (77.65%) was significantly higher than infection rate in urban 90 (87.26%), this is in agreement with Al-Quraishi in Babylon [18], (probably because of the difference between the city and countryside in lifestyle and vaginal infection mainly affecting people living in poor or disadvantaged communities [18,19]. In parity, the infection percent increase in multipara status had the highest rate 85 (89.47%) followed by unipara status 49 (75.38%), and finally the nullipara status 22 (55%). It appeared that infection with is strongly correlated with reactivation of some agents, abortion, through vaginal delivery and vaginal hygiene practices (such as douching) and type of contraceptive this result agreement with [20].

In the current study, the majority of symptoms were malodor and profuse discharge than the vulvar itching, other symptoms occur with less frequency.

Symptoms alone are not sufficient to make reliable diagnosis of sexually transmitted pathogen. Symptoms in women with malodor signs represents 47 (85.45%). Profuse discharge, vulvar itching, joint pain, dysuria and cervical abnormalities were represented 84.91% (45/53), 57.78% (26/45), 75% (15/20), 93.33% (14/15), 75% (9/12) respectively. It is known that vulvovaginitis is characterized by discharge and strong odor in particular which have bacterial vaginosis while *T. vaginalis* is associated with dysuria and pain.

The pathogenesis of infection leading to the most aforementioned symptoms is somehow obvious; however, the relationship between this infection and symptoms is not so clear. It is believed that the adhesion of STPs to vaginal epithelial cells (VECs) plays an important role in the pathogenesis STPs. Han et al. showed that the inflammatory mediators made by VECs in response to *T. vaginalis* activate and attract masts cells and neutrophils, thus, joint pain, especially in knees and lower back, can be used as indicator for *T. vaginalis* [21-23].

Presence of cervical abnormalities in infected patients agree with the study of Donders et al. 2013 that *T. vaginalis* infection is also associated with the cervical cytological abnormalities [24] and depicted as a risk factor for cervical dysplasia and cancer [25].

In this study, most women who tested positive for the evaluated disorders reported some clinical signs, being discharge the most prevalent one. However, this sign was statistically associated with any infection, unlike what was reported by Gama [5]. No association between the occurrences of strong odor and the three infections was found, as well as no positive correlation for dysuria with vaginitis, corroborating other studies.

The disparity in the prevalence of STPs could be attributed to many reasons; environmental and socio-economic factors, accurate take of sample form patient by gynecologist, diagnostic method, number of tested samples, type of samples and cultural factors.

Diagnosis of *G. vaginalis* were done by demonstration of clue cell by wet mount, gram stain and culture.

The infection rate of *G. vaginalis* wet preparation technique and Gram's stain 95(47.5%), Gram's stain along with culture methods help to demonstrated the causative agent of bacterial vaginosis, this result agrees

with reports done by Amsel et al (1983) [26] whom they reviewed that the prevalence of clue cells on wet mount preparation of *G. vaginalis* discharge and demonstration that there was a strong inverse relationship between the bacterial vaginosis and *G. vaginalis* infection. It is commonly believed as with many studies done by Gardner et al. (1957) [27]. These previous studies found that Gram's stain method represent the optimal laboratory test for of diagnosis of bacterial vaginosis, which is simple to perform, sensitive and specific. All these studies are in agreement with our study that shows *G. vaginalis* is the most prevalent form of vaginal disturbances in women. For *T. vaginalis*, the result in this study was higher than results in different provinces in Iraq. In Baghdad, 7% [28] while in Najaf 10.88% [15].

This study showed that *G. vaginalis* was isolated from (50%) of infection cases using culture method. Isolation of *G. vaginalis* from culture of vaginal swabs are unreliable and should not be utilized for diagnosis because of the associated of various anaerobes and presence of these organisms in normal vaginal flora, this result of positive culture cannot be considered diagnostic for this infection because when the predictive value of positive *G. vaginalis* culture is around (54%) [29].

Vaginal culture is one of the most difficult cultures to be evaluated in a clinical microbiology practice. The necessity of some expensive and complicated processes for diagnosis of some specific agents, age related variability of normal vaginal flora, and failure to make a diagnosis caused by the temporary presence of some pathogens in normal flora can be listed among the probable causes of that problem [30].

Regarding to the RT-PCR test, that from 200 samples, only 156/200 samples were diagnosis to be infected by RT-PCR. This assay detected 120 (60%) cases of *G. vaginalis*, 34 (17%) cases of *T. vaginalis*. PCR is more accurate to detect the STPs. Molecular methods are considered the gold standard for diagnosis, given the excellent sensitivities and specificities in diagnosis. because it is allowed to distinguish between STPs However, new molecular methods such as PCR, qualitative and quantitative real time PCR rapid detection were STDs in comparison to serological methods have been used as common. Moreover, monitoring of DNA level of a pathogen in body fluids can reveal the status of the disease, its response to medication, and its resistance patterns [31,32].

Regarding to age, the study showed that STPs occurred in reproductive age group 15-54 years this may be attributed to the reality that the sexually active woman, menstrual cycle, hormonal factors and use of contraceptive. Women in reproductive aged group were at an increased risk for vaginitis. The highest rate of infection with G.V. among 15-36 years old, this finding is in agreement with other study [32], but in contrast to other study that showed marked increase in the prevalence of *G. vaginalis* with increasing age [33].

They reported high prevalence of *T. vaginalis* among age group 15-24 years of age followed by 25-34 and 35-44 years, the lowest in 45-54 years of age, this is identical with the study of that proved the prevalence of T. vaginalis was higher in women over age 15 years [34,35].

In previous study in Baghdad, almost close percentage for those women 12% (12/100) [36]. *T. vaginalis* found that infection predominantly occurs in age group 15-24 years represent the highest rate and this is in agreement with our study, Omer et al. (1985) in Sudan, found that *T. vaginalis* infection predominantly occurs in age group 16-19 years, this result agreement with our study in which we found that the lowest rate occur in age group 16-27 years [37].

This result may be due to the ability of the parasite to alter at the vaginal environment for its survival. In the present study, the distribution of microorganisms was high among different age groups. A study done by Mahdi et al. showed that the infection rate (12.6%) was found in women of reproductive age. The majority of positive cases were in the age

group 20-40 years, while women near or post-menopause showed low incidence of infection, and this is probably due to absence of suitable environment for growth of *T. vaginalis*. Vaginal infection is considered one of the major feminine health problems [38] from time to time during their reproductive lives due to its strong relationship with the menstrual cycles, birth control methods, aging, medicines, or changes after pregnancy [39,40].

Most common co-infection is between *G. vaginalis* cases and *T. vaginalis* were 30/156 (19.23%) cases of co-infection. Statistically, there were highly significant differences among co-infection cases. All these findings suggest that there are combined infections in patients, which in agreement with our study. Several workers studied the association of *T. vaginalis* with other pathogens many of them agreed that *T. vaginalis* may precipitate other type of infection which may be due to sexual risk behavior. The variable discharges that noticed in this study might be resulted from co-infection of *T. vaginalis* with other microbial pathogens. Many studies found combined infections, found *T. vaginalis* and *G. vaginalis*. *G. vaginalis* was related to concurrent infection with *T. vaginalis*. The highest rate of combined infection was seen in cases infected with both *T. vaginalis* and *G. vaginalis* since both favor the growth in similar environment specially pH >4.5. *T. vaginalis* might be responsible for the change in normal vaginal flora and may therefore precipitate for *G. vaginalis* bacterial vaginosis [41-44].

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Authors Contribution:

Ali conducted the sampling, isolation, and staining, the molecular work and writing the manuscript. Dr. Al-Marsome and Dr. Almoayed supervised the work, edit and finalize the writing of the study.

Conflict of interest

The authors declare no conflict of interest.

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Correspondence to Mariam k. Ali
E-mail: mariam.kareem25@yahoo.com
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Remarkable Enhancement of Mean Platelet Volume in Iranian patients with Type 2 Diabetes Mellitus with no Dependence on Hemoglobin A1c Level

Hojjat Khalili-Hezarjaribi[1] *PhD,* **Shima Mahdavian**[1] *MD*, **Marjaneh Mirsadraei**[1] *MD*, **Roya Farahmand-Bovanlou**[2] *MSc*, **Ali Jahanbazi Jahanabad**[3] *PhD*

[1]Research and Education Dept., Reza Radiotherapy and Oncology Center, Mashhad, Iran, [2]Dept. of Biology, Payame Noor University of Mashhad, Iran, [3]Dept. of Biochemistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background Diabetes mellitus (DM) is a pandemic disease that leads to several complications. Platelet reactivity plays a major role in diabetes complications. Mean platelet volume (MPV) is a marker of platelet size that is easily determined on routine automated blood counters. Studies have been found that MPV is enhanced in patients with DM.

Objective To assess any relationship between MPV in type 2 Diabetic persons and with glycohemoglobin (HbA1c) level.

Methods The study included a total of 130 subjects who were referred to Samenol Aemme and Mehregan hospitals in Mashhad city, Iran, between April 2013 and November 2014 for a routine check-up. All subjects were divided into two groups as following: Patients with type 2 diabetes group, which comprises 63 subjects and non-diabetic control group, which comprises 57 individuals. Blood sample from each individual was collected after a 12-hour overnight fasting. MPV, HbA1c and fasting blood sugar (FBS) were also monitored.

Results MPV values were found to be 8.7±0.36 fl ($p<0.001$) and 9.8±0.42 fl ($p<0.001$) for control and study groups respectively. FBS values were found to be 95.9±10.3 mg/dl ($p<0.001$) and 202.8±5.1 mg/dl ($p<0.001$) for control and study groups respectively. MPV values were found to be 9.8±0.28 fl and 9.8±0.46 fl ($p=0.813$) for diabetic subgroups with HbA1c <7 and HbA1c ≥7 respectively.

Conclusion The current study showed that: i. MPV was enhanced considerably in patients with DM in contrast to healthy controls. ii. Its increase was not dependent on glycemic control reflected by HbA1c.

Keywords Platelet reactivity, HbA1c measurement, diabetic patients.

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List of abbreviations: DM = Diabetes Mellitus, FBS = Fasting blood glucose, HbA1c = Hemoglobin A1c, MPV = Mean platelet volume

Introduction

Diabetes Mellitus (DM) is an important health problem worldwide and it has been known as the most common endocrine disorder [(1)]. By the year 2025, it is estimated that more than 300 million people worldwide will have DM and by 2030 this would have risen to 552 million [(2-5)]. As the disease progresses, severe diabetic complications will be occurred such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration [(6-8)]. According to previous studies, type 2 DM

and it's related conditions are associated with subclinical inflammation [9-10]. This systemic inflammation may be contributed to platelet reactivity observed in patients with type 2 DM [11]. Platelets play a major role in the normal hemostasis. The large platelets contain more dense granules are more potent than smaller platelets and hence more thrombogenic [12]. Some studies have been shown that acute hyperglycemia results in increased platelet activation [13]. Fasting blood glucose (FBS) and hemoglobin A1c (HbA1c) are parameters widely used to monitor glycometabolic control in patients with DM. HbA1c is a more useful marker to determine mean blood glucose levels over a long-time period [14]. Some studies have been found that levels of FBS and HbA1c are significantly correlated with expression of markers of platelet activation [15-17]. Regarding to the fact that increased morbidity and mortality in type 2 DM are associated with macrovascular (cardiovascular diseases, stroke, and peripheral arterial disease) and microvascular (nephropathy, neuropathy and retinopathy) complications due to platelet dysfunction, the importance of study of platelets is considerable [18-21].

Platelet volume is a marker of platelet activation and function and is measured as mean platelet volume (MPV). MPV is emerging as a new risk factor for vascular complications of DM of which atherothrombosis plays a crucial role [22]. Although several measurements of platelet activity have emerged, many of these measurements are time-consuming, expensive, use a high sample volume, or require specialty training. In contrast, MPV is a marker of platelet size that is easily determined on routine automated blood counters and routinely available at a relatively low cost [23]. MPV was found to be significantly higher in diabetic patients [24,25]. Also, some studies have been shown that in patients with DM, higher MPV correlates with higher HbA1c level [26]. On the other hand, another study has been found that MPV increases independent of HbA1c level [10]. At the present, it is unclear whether MPV is related to FBS, and HbA1c and there is a range of different data about this issue in the literature [30]. Hence, in the current study it was aimed to assess MPV behavior in patients with DM and also evaluate the correlation between HbA1c and MPV in Iranian patients with type 2 DM.

Methods

Study population and sample:

In the current study, a total of 130 subjects were categorized into two groups. One group of diabetic patients and other include non-diabetic controls. All participants under study referred to Samenol Aemme and Mehregan hospitals in Mashhad city, Iran, between April 2013 and November 2014 for a routine check-up. Blood sample from each individual was collected into two types of tubes after a 12-hour overnight fasting as following: non-coagulated blood sample collected in tube containing EDTA, as anticoagulant reagent, was used to assess MPV index and HbA1c Levels and coagulated blood sample in a plain tube without EDTA for evaluation of FBS [27]. The venous blood samples were tested within 30 minutes of collection to minimize variations due to sample aging. MPV was measured using an automatic blood counter system (KX-21 sysmex, Japan).

Also, FBS and HbA1c levels were measured by laboratory standard methods using BT-3000 auto analyzer system, Italy. All obtained data were recorded. Patients with iron deficiency anemia, hypo-hyperthyroidism, congestive heart failure, recent infection were excluded [10]. For the sake of minimizing confounding factors, patients with leukocytosis, anemia or thrombocytopenia were not included as they may affect platelet and erythrocyte size. Patients with known inflammatory conditions such as rheumatoid arthritis, systemic lupus erythematosus, were also excluded [10]. In order to evaluate the correlation between MPV and HbA1c level, then, patients with DM were divided into two groups on the basis of HbA1c

<7 and HbA1c ≥7 and obtained results were recorded [10,26].

Data analysis

Data was assessed by using SPSS program. (SPSS 15.0; SPSS Inc., Chicago, IL, USA). Results expressed as mean±SD. Variables are conducted with either independent samples t test (for normal distributed variables) or Mann-Whitney U test (for abnormal distributed variables). A p value of < 0.05 is considered as statistically significant [10].

Results

The study included a total of 130 subjects who were divided into two groups as following: type 2 diabetic Patients group that comprises 63 subjects (25 males and 38 females) and control group that comprises 57 subjects (٣٢ males and 35 females). Table 1 showed age and gender of both control and study groups. No difference between non-diabetic and diabetic individuals statistically were seen.

Table 1. Demographic characteristics of the diabetic patient and non-diabetic control groups, presented as mean±SD

	Non-diabetic Control Group			Diabetic patients			P value
	Total	Male	Female	Total	Male	Female	
No. of Subjects	67	32	35	63	25	38	0.065
Mean Age	56.91±9.9	56.5±10.2	57.3±9.9	59.41±10.3	58.3±11.8	60.1±9.4	

Figure 1 Compares FBS by autoanalyser and HbA1c blood sugar values between non-diabetic control and type 2 diabetic patient groups and it revealed a statistical significant difference between both groups (p<0.05).

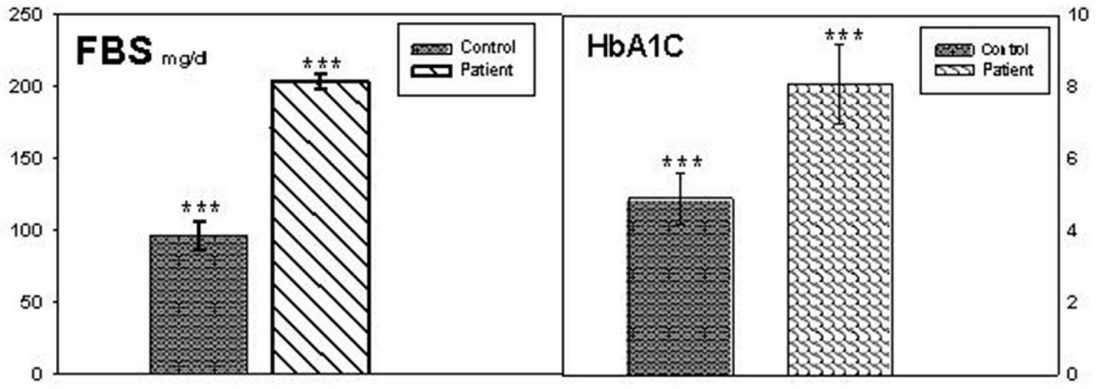

Figure 1. Comparison of FBS and HbA1c blood sugar values between non-diabetic control and type 2 diabetic patients. (p<0.001)

Regarding to the pooled results, FBS and HbA1c values are considerably greater in study groups compared to controls as was expected. This study showed that the MPV was significantly higher in diabetic patients compared to non-diabetic controlled subjects (p<0.001) (Figure 2).

Furthermore, by regrouping of diabetic patients according to HbA1c blood sugar level whether < 7% or ≥ 7% and by comparing the MPV in both groups, no statistical difference was found between those two groups. However, there was statistical significant difference in FBS between the two HbA1c group (figure 3).

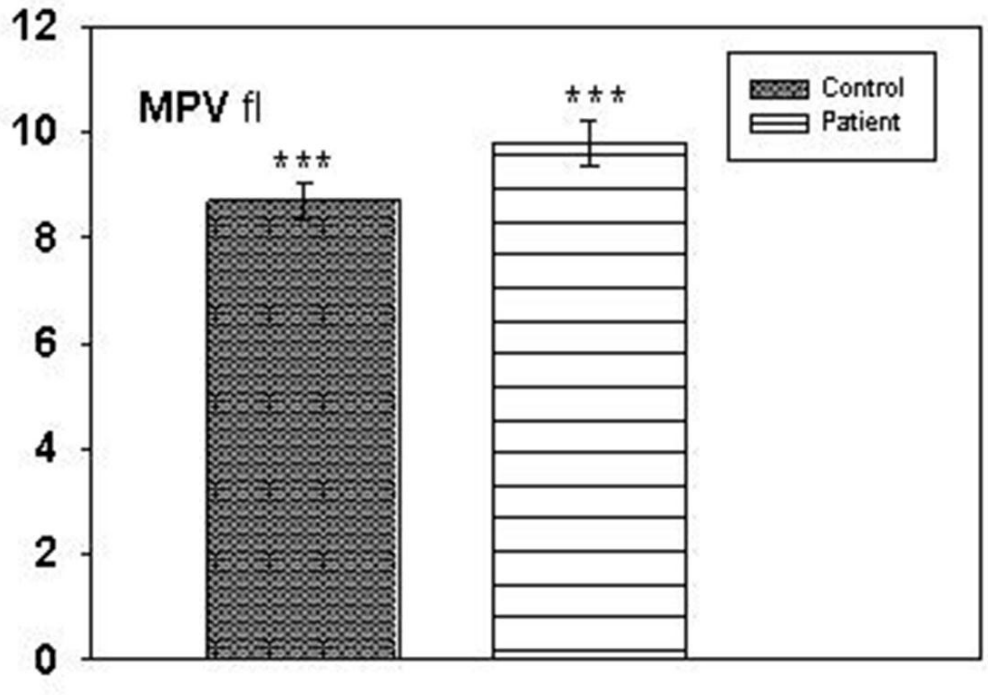

Figure 2. The MPV level in non-diabetic control and type 2 diabetic patient groups, based on estimation FBS by autoanalyzer (p<0.001)

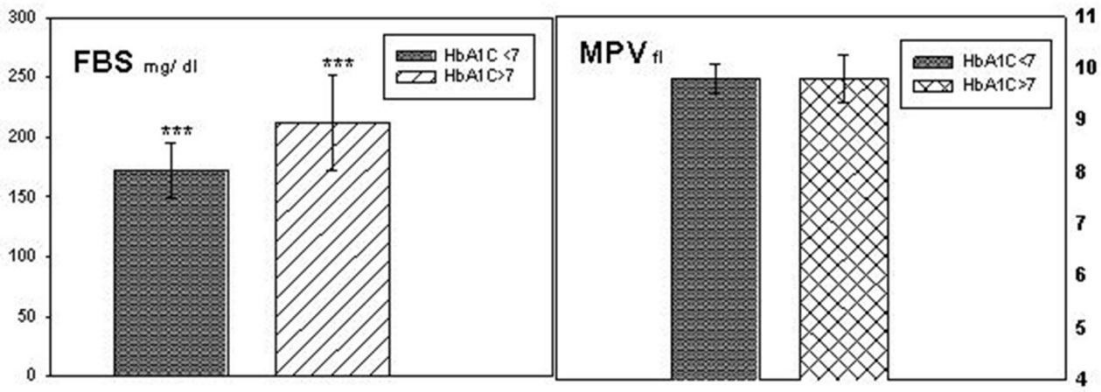

Figure 3. Comparison of MPV values between diabetic subjects based on HbA1c value

Discussion

DM has been known as a pandemic disorder [1]. Many studies have been shown DM as a prothrombic state because platelets function and morphology are usually altered during this disease [28]. Several studies have been shown positive correlation between MPV and DM and also MPV and HbA1c level [26,28]. On the other hand, some studies show that MPV enhancement is consequence of diabetes

complications such as inflammation and no correlation have been found between elevated MPV value and HbA1c level [10]. In the current study, MPV, as one of the important markers of platelet activity, was assessed to understand it's behavior better during DM. According to the obtained results, there was a remarkable enhancement of MPV value in diabetic patients compared to non-diabetic healthy controls. This is inconsistent with majority of previous studies. On the other side, while comparing MPV value based on HbA1c level, no considerable differences have been observed between two groups with HbA1c <7 and HbA1c ≥7. However, when blood sugar was estimated by autoanalyser the MPV was significantly higher in HbA1c ≥7 group compared to HbA1c <7 group. Therefore, it can be concluded that MPV is increased during DM independent of HbA1c level. This is inconsistent with the results obtained by some studies which have shown positive relationship between elevated MPV and HbA1c level [26,28]. According to the previous literatures, higher MPV is related to higher platelet activity. Multiple mechanisms caused by metabolic and cellular abnormalities have been suggested to play a role in the increased platelet activity observed in patients with DM [10,28]. Among them hyperglycemia and insulin resistance can be addressed as the majors and lead to higher platelet activity. Platelets from patients with type 2 DM have increased expression of adhesion molecules and activation markers [28]. Also, a positively significant correlation between platelet activation markers and level of HbA1c has been observed [28]. On the other side, regarding to the obtained results in the present study, it seems that MPV increases independent of HbA1c level. It can be explained in this way that long term glycemic controls may indirectly affects platelet activity, however, there are far more unknown parameters affecting platelet activity in patients with DM. This is an observational study with relatively low size of samples. In order to understand more about molecular mechanisms affecting the platelet activity and consequently MPV value in DM, more studies with greater range of sampling are needed.

According to the obtained results in the current study, it we can be concluded that: i. MPV was significantly increased in patients with DM in contrast to healthy controls. ii. MPV increases independent of HbA1c levels in patients with DM.

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Authors Contribution:

Dr. Khalili-Hezarjaribi developed the original idea and designed the assessment protocol, wrote the manuscript. Dr. Mahdavian, Dr. Mirsadraei and Dr. Farahmand-Bovanlou collect data and analyzed them and prepared the manuscript. Dr. Jahanabad contributed in the data collection.

Conflict of interest

The authors declare no conflict of interest for the present study.

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Correspondence to Dr. Hojjat Khalili-Hezarjaribi
E-mail: Hojjatkhalili@RROC.IR
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Prevalence and Determinants of Depression Among Traumatic Spinal Cord Injured Patients Attending Ibn-Al-Quff Hospital, Baghdad, Iraq

Shalan J.R. Al Abbudi[1] *FIBMS Psych,* **Khalida I. Ezzat**[2] *PhD*, **Mohammed S. Farhan**[2] *MSc*, **Ali A. Zebala**[2] *MSc*, **Mohammad S.J. Al-Beedany**[3] *D Prosthestic Orthosis*, **Delkhwaz J. Hamdy**[1] *BSc*

[1]Section of Psychiatry, Dept. of Medicine, Imamein Kadhimein Medical City, Baghdad, Iraq, [2]Ibn-Rushd Mental Hospital, Baghdad Iraq, [3]Hakeem Hospital, Baghdad, Iraq.

Abstract

Background Depression is a common consequence of spinal cord injury.

Objective To identify the prevalence and potential risk factors of depression among spinal cord injured inpatients, and assessment of the severity of depression.

Methods A cross-sectional study conducted at Ibn Al Quff Hospital for spinal cord injury rehabilitation. All inpatients with traumatic spinal cord injury were recruited excluding severely injured and those injured due to congenital and medical causes. Socio-demographic variables, spinal cord injury characteristics and comorbidity were compiled. Self-Reporting Questioner (SRQ-20) was used to identify mental symptoms. DSM-IV criteria for depression and Hamilton-17 Scale, for assessment of severity of depression were used.

Results A total of 274 spinal cord injured inpatients were approached; 93% responded; Paraplegics 75.7% and tetraplegics 24.3%. Violence was the major cause of injury. Seventy four percent (74.1%) had depression; 44% of them had severe and very severe depression. Depression was significantly associated with age (P=0.001), gender (P=0.001), education level (P=0.038), occupation (P=0.003); smoking habit (P=0.035), duration of injury (P=0.003), times of admission (P=0.000), and comorbidity (P=0.018).

Conclusion prevalence of depression is high and frequent among spinal cord injured inpatients. Demographic and spinal cord injury variables are significantly associated with depression and are the most important determinants of depression.

Keywords Depression, spinal cord, violence, prevalence, Iraq.

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List of abbreviations: DSM-IV = Diagnostic and Statistical Manual of Mental Disorders-IV, HAM-D = Hamilton scale for severity of depression, MDD = Major depressive disorder, SCI: Spinal cord injury, SRQ = Self-reporting questionnaires, WHO = World health organization.

Introduction

Spinal cord injury (SCI) is a devastating condition causing profound life changes for millions of people around the world [(1)]. Over 80% of traumatic SCI are male, with an average age at injury s of about 40 years and most frequent causes of injuries include motor vehicle accidents, violence, falls, and recreational accidents [(2)]. SCI typically causes paralysis and permanent disability. Despite costly and aggressive rehabilitative options, injuries to the spinal cord remain permanent and create lifelong challenges for survivors [(1)].

SCI results in diminished mobility, greatly reduced functional independence, and difficulties with socialization and employment [3]. Exposure to life-threatening conditions or severe mental stress may lead to various psychological reactions including depression. One of the deleterious stresses is that experienced during war. Veterans encumbered with physical disabilities are more prone to depression, among other psychological disorders [4]. During the Iraq-Iran war, many young soldiers and para-military troops sustained physical disabilities which were compounded by psychological conflicts [5]. The impact of SCI on psychological status has been variously debated. Several studies have suggested that SCI is associated with raised risks of psychological problems. Negative psychological states have been found in 30-40% percent of patients with SCI [6-8]. The Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) [9] defines many disorders including major depressive disorder (MDD) on the basis of the presence of a minimum number of symptoms or features from a list [10,11]. Depression is the most common psychological issue associated with SCI [4], reportedly affecting approximately 30% of patients, and is generally characterized by depressed mood and diminished pleasure over a two-week span accompanied by issues including energy loss, concentration difficulties, and sleep or appetite disturbances [9]. There is a large body of literature documenting the high prevalence of depression, psychological distress, and psychological morbidity after spinal cord injury (SCI) [12-14]. In a recent study of community-residing people with traumatic SCI, the rate of probable major depression was found to be 3 times that of the general population [12,15].

In Iraq, Ibn Al-Quff hospital for spinal cord injuries was opened on October 1982 after increased incidence of spinal cord injuries at beginning of Iraq-Iran war [16,17]. More than four thousand spinal cord injured (SCI) patients had been rehabilitated in Iraq during the last three decades at Ibn Al-Quff hospital spinal cord injury rehabilitation [18]. About 84.8% of spinal cord injured persons were paraplegic, and about 15.2% were quadriplegic persons, 90.5% male and 9.5% were female. The causes of SCIs, are approximately 50% for high velocity missiles, 18% road traffic accidents, 16% fall from height, 6% stab wound, and 10% for others. A total of 1768 spinal cord injured persons were admitted to the Ibn Al-Quff hospital during 2003-2010 [19].

This study aimed to through a light on the prevalence of depression among physically disabled traumatic spinal cord injured patients at Ibn Al Quff spinal cord injuries hospital, Baghdad, Iraq, and assessment of the severity of depression.

Methods

Design and setting

This is a cross-sectional study with analytic component. It was conducted in Ibn Al Quff hospital for spinal cord injuries, Baghdad, Iraq. The data collection was done during the period from June 1st, 2011 to November 1st, 2012.

Study population and sampling

All inpatients with traumatic spinal cord injury, both genders were included.

Inclusion criteria: All traumatic spinal cord injured patients with paraplegia or quadriplegia, aged ≥18 years, of both sexes, and accepted to participate and have the interview.

Exclusion criteria: Severe injured patients, who cannot respond to questions, age <18 years, with paraplegia or quadriplegia by other non-traumatic causes like medical disorder or congenital disorders, and those with substance abuse.

Data collection tools: Basic socio-demographic variables, spinal cord injury history and history of co morbid characteristic and complications were compiled using a questionnaire filled through a direct interview. Mental status of the traumatic spinal cord injured inpatient was assessed using Self-Reporting Questionnaires Scale (SRQ-20) that was developed by the WHO

and used in many countries. According to previous studies conducted in Iraq, the cut-off point identified used to categories "potential psychiatric cases" and more generally persons with significant psychological distress was seven [20]. Those with positive SRQ-20 results were assessed for the presence of depression using the DSM-IV criteria [9]. Those with "depression" were further assessed for the severity of depression using the Hamilton scale. It contains 17 items to be rated (HAM-17) [21].

Definition of variables: The independent variables evaluated to explain depression were socio-demographics (age, gender, marital status, level of education, occupation, smoking habits, characteristics of the disability (types, causes, duration, admission times, and rehabilitation), complications and bed sores, and comorbid condition.

Statistical analysis

SPSS version 17 used for data entry and analysis. The prevalence of depression and its 95% confidence interval was calculated. Univariate analysis using Chi square was applied to identify potential risk factors of depression.

Ethical Issues

Informed consent was obtained from the patients after clarifying the objectives of the study. Names were kept anonymous and interviews were conducted with full privacy.

Results

A total of 274 spinal cord injured inpatients were approached; 255 welcomed and accepted to participate (response rate: 93%). Paraplegics were 193 (75.7%) and quadriplegics were 62 (24.3%). Distribution by sociodemographic and spinal cord injury characteristics and co-morbid conditions are shown in tables 1 and 2.

The prevalence of depression was 74.1%. About 44.7% of the sample has severe and very severe depression. None were receiving treatment; psychotherapy or medications. A cross classification of patients with and without depression by socio-demographic and spinal cord injury patients' characteristics and co-morbid conditions is shown in table 3 and 4.

The degree of severity of depression was explored according to socio-demographic characteristics and other co morbid features associated with spinal cord injury shown in table 5 and 6.

The prevalence of depression was highest among those aged (46-55) years (91.66%) and lowest among those aged 26-35 years (52.17%) (P=0.001). Females had significantly higher proportion of depression (94.12%) compared to males (69.12%) (P=0.001). Depression was significantly higher among illiterate (90%) than other educated patients (P=0.038). Unemployed (85.7%) and housekeepers (92.2%) patients had significantly higher prevalence of depression than employed patients (66.6%) (P=0.003). Depression was significantly higher among smokers (83.3%) than non- smokers (P=0.035).

The prevalence of depression was not significantly different by marital status (P=0.258), types of disability (P=0.727), and causes of spinal cord injury (P=0.086). The prevalence of depression was highest among those with injury of spinal cord for 1-5years duration (90.6%) (P=0.003), those with frequent admissions to the hospital (89.3%) (P=0.001), those who have other comorbid illnesses (P=0.018), and among those exposed to life events (P=0.008).

The prevalence of depression was not significantly affected by family history of mental illness (P=0.116), duration of admission (P=0.744), accompanied persons (P=0.688), visitors (P=0.646), rehabilitation (P=0.434), walking aids (P=0.935), complications (P=0.253), and presence of bed sores (P=0.324).

The assessment of depressed spinal cord injured patients by severity of depression revealed that 25.9% had mild depression, 11.8% had moderate depression and 44.7% had severe or very severe depression (table 5 and 6). The severity of depression was significantly associated with socio-demographic characteristics; age of the patients (P=0.000), gender (P=0.000), marital status (P=.029),

education level (P=0.000), occupation (P=0.000), and smoking habit (P=0.000).
The severity of depression was significantly associated with the causes of spinal cord injury (P=0.010), duration of injury (P=0.011), times of admission (P=0.000), complications (P=0.001), and co-morbidity (P=0.015).

Table 1. Distribution of the study group by sociodemographic characteristics and smoking habit

Socio demographic characteristic		Spinal Cord Injury		Total (255)	
		Paraplegia	Tetraplegia	No.	(%)
Age Group	18-25 yrs	70	23	93	36.5
	26-35 yrs	51	18	69	27.1
	36-45 yrs	33	9	42	16.5
	46-55 yrs	28	8	36	14.1
	56-65 yrs	11	4	15	5.9
Sex	Male	161	43	204	80
	Female	32	19	51	20
Marital Status	Single	90	36	126	49.4
	Married	98	25	123	48.2
	Divorced	5	1	6	2.4
Education Level	Illiterate	19	11	30	11.8
	Primary	92	31	123	48.2
	Intermediate	50	13	63	24.7
	Secondary	18	6	24	9.4
	institute and college	14	1	15	5.9
Occupation	Unemployed	20	1	21	8.2
	Employed	16	2	18	7.1
	free work	85	35	120	47.1
	house wife	23	16	39	15.3
	Military	23	4	27	10.6
	Retired	3	0	3	1.2
	Student	23	4	27	10.6
Smoking	No	137	46	183	71.8
	Yes	56	16	72	28.2

Table 2. Distribution of the study group by spinal cord injury characteristics and co-morbid conditions

		Spinal Cord Injury		Total (255)	
		Paraplegia	Quadriplegia	No.	(%)
Cause of injuries	Bullet	28	12	40	15.7
	Shell Explosion	48	41	89	34.9
	FFH	45	5	50	19.6
	RTA	72	4	76	29.8
Duration of Injury	Less than 1 year	104	27	131	51.4
	1-5 years	46	7	53	20.8
	More than 5 years	43	28	71	27.8
Duration of Admission	Less than 1 month	14	7	21	8.2
	1-6 months	148	47	195	76.5
	More than 6 months	31	8	39	15.3
Times of Admission	First Admission	133	38	171	67.1
	Frequent Admissions	60	24	84	32.9
Accompanied Persons	Absent	33	18	51	20
	Present	160	44	204	80
Visitors	Absent	20	7	27	10.6
	Present	173	55	228	89.4
Rehabilitation	Absent	26	4	30	11.8
	Present	167	58	225	88.2
Walking Aids	Absent	42	13	55	21.6
	Present	151	49	200	78.4
Complications	Absent	81	27	108	42.4
	Present	112	35	147	57.6
Co-morbidity	Absent	185	55	240	94.1
	Present	8	7	15	5.9
Pressure sore	Absent	131	30	161	63.1
	Present	62	32	94	36.9

Table 3. Distribution of the study group by depression and the socio-demographic characteristics

		Depression				Total (255)		P value
		Not Depressed		Depressed				
		No.	(%)	No.	(%)	No	(%)	
Age Group	18-25 yrs	21	22.6	72	77.4	93	36.47	0.001
	26-35 yrs	33	47.83	36	52.17	69	27.05	
	36-45 yrs	6	14.29	36	85.71	42	16.47	
	46-55 yrs	3	8.34	33	91.66	36	14.11	
	56-65 yrs	3	20	12	80	15	5.88	
Sex	Male	63	30.88	141	69.12	204	80	0.001
	Female	3	5.88	48	94.12	51	20	
Marital Status	Single	36	28.57	90	71.43	126	49.41	0.258
	Married	30	24.39	93	75.61	123	48.23	
	Divorced	0	0	6	100	6	2.35	
Education Level	Illiterate	3	10	27	90	30	11.76	0.038
	Primary	42	34.14	81	65.86	123	48.23	
	Intermediate	12	19.05	51	80.95	63	24.7	
	Secondary	6	25	18	75	24	9.41	
	College+	3	20	12	80	15	5.88	
Occupation	Unemployed	3	14.28	18	85.72	21	8.23	0.003
	Employed	6	33.33	12	66.67	18	7.05	
	free work	30	25	90	75	120	47.05	
	house keeper	3	7.69	36	92.2	39	15.29	
	Military	12	44.44	15	55.56	27	10.58	
	Retired	0	0	3	100	3	1.17	
	Student	12	44.44	15	55.56	27	10.58	
Smoking	No	54	29.5	129	70.5	183	71.76	0.035
	Yes	12	16.67	60	83.33	72	28.23	
Total		66	25.88	189	74.12	255	100%	

Table 4. Distribution of the study group by depression and spinal cord injury characteristics and presence of complication or comorbid condition

		Depression				Total (255)		P value
		Not Depressed		Depressed				
		N0.	%	No.	%	No.	%	
Disability	Paraplegia	51	26.4	142	73.6	193	75.7	0.727
	Quadriplegia	15	24.2	47	75.8	62	24.3	
Cause of injuries	Bullet	4	10	36	90	40	15.7	0.086
	Shell Explosion	25	28.1	64	71.9	89	34.9	
	FFH	16	32	34	68	50	19.6	
	RTA	21	27.6	55	72.4	76	29.8	
Duration of injury	Up to 1 year	44	33.6	87	66.4	131	51.37	0.003
	1-5 years	5	9.4	48	90.6	53	20.8	
	More than 5 years	17	23.9	54	76.1	71	27.8	
Duration of admission	Less than 1 month	5	23.8	16	76.2	21	8.2	0.744
	1-6 Months	49	25.1	146	74.9	195	76.5	
	More than 6 months	12	30.8	27	69.2	39	15.3	
Times of Admission	First Admission	57	33.3	114	66.7	171	67.1	0.000
	Frequent Admissions	9	10.7	75	89.3	84	32.9	
Accompanied persons	No	12	23.5	39	76.5	51	20	0.688
	Yes	54	26.5	150	73.5	204	80	
Visitors	No	6	22.2	21	77.8	27	10.6	0,646
	Yes	60	26.3	168	73.7	228	89.4	
Rehabilitation	No	6	20	24	80	30	11.7	0.434
	Yes	60	26.7	165	73.3	225	88.2	
walking aids	No	14	25.5	41	74.5	55	21.6	0.935
	Yes	52	26	148	74	200	78.4	
Complication	No	24	22.2	84	77.8	108	42.4	0.253
	Yes	42	28.6	105	71.4	147	57.6	
Bedsores	No	45	28	116	72.	161	63.1	0.324
	Yes	21	22.3	73	77.7	94	36.9	
Co morbidity	No	66	27.5	174	72.5	240	94.1	0.018
	Yes	0	0	15	100	15	5.9	
Total		66	25.88	189	74.12	255	100	

Table 5. Distribution of the study group by degree of severity of depression and the socio-demographic characteristics

		Depression										Total (255)		P value
		No Depression		Mild Depression		Moderate Depression		Severe Depression		Very Severe Depression				
		No	%	No	%	No	%	No	%	No	%	No.	(%)	
Age Group	18-25 yrs	21	22.5	3	3.2	21	22.5	18	19.3	30	32.2	93	36.5	0.000
	26-35 yrs	33	47.8	6	8.7	9	13.05	9	13.05	12	17.4	69	27.1	
	36-45 yrs	6	14.3	9	21.43	6	14.3	6	14.3	15	35.7	42	16.5	
	46-55 yrs	3	8.3	6	16.7	9	25	3	8.3	15	41.7	36	14.1	
	56-65 yrs	3	20	6	40	0	0	0	0	6	40	15	5.9	
Sex	Male	63	30.9	27	13.2	42	20.6	24	11.8	48	23.5	204	80	0.000
	Female	3	5.9	3	5.9	3	5.9	12	23.5	30	58.8	51	20	
Marital Status	Single	36	28.6	9	7.1	27	21.4	18	14.3	36	28.6	126	49.4	0.029
	Married	30	24.4	18	14.6	18	14.6	18	14.6	39	31.7	123	48.2	
	Divorced	0	0	3	50	0	0	0	0	3	50	6	2.4	
Education Level	Illiterate	3	10	3	10	3	10	3	10	18	60	30	11.8	0.000
	Primary	42	34.1	12	9.8	30	24.4	15	12.2	24	19.5	123	48.2	
	Intermediate	12	19.05	9	14.3	12	19.05	9	14.3	21	33.3	63	24.7	
	Secondary	6	25	6	25	0	0	6	25	6	25	24	9.4	
	College+	3	20	0	0	0	0	3	20	9	60	15	5.9	
Occupation	Unemployed	3	14.3	0	0	0	0	6	28.6	12	57.14	21	8.2	0.000
	Employed	6	33.3	3	16.7	0	0	0	0	9	50	18	7.1	
	Free work	30	25	15	12.5	36	30	15	12.5	24	20	120	47.1	
	house keeper	3	7.7	3	7.7	3	7.7	9	23.1	21	53.9	39	15.3	
	Military	12	44.4	3	11.1	6	22.2	3	11.1	3	11.1	27	10.6	
	Retired	0	0	3	100	0	0	0	0	0	0	3	1.2	
	Student	12	44.4	3	11.1	0	0	3	11.1	9	33.3	27	10.6	
Smoking	Not smoker	54	29.5	24	13.1	15	8.2	24	13.1	66	36.1	183	71.8	0.000
	Smoker	12	16.7	6	8.3	30	41.6	12	16.7	12	16.7	72	28.2	
Total		66		30		45		36		78		255	100	

Table 6. Distribution of the study group by degree of severity of depression and spinal cord injury characteristics and presence of complication or comorbid condition

		Depression												P value
		No Depression		Mild Depression		Moderate Depression		Severe Depression		Very Severe Depression		Total (255)		
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Cause of injuries	Bullet	4	10	1	2.5	6	15	13	32.5	16	40	40	15.7	0.010
	Shell Explosion	25	28.1	15	16.9	14	15.74	8	8.99	27	30.3	89	34.9	
	FFH	16	32	5	10	10	20	3	6	16	32	50	19.6	
	RTA	21	27.6	9	11.85	15	19.7	12	15.79	19	25	76	29.8	
Duration of injury	Less than 1 year	44	33.6	15	11.5	21	16.03	21	16.03	30	22.9	131	51.4	0.011
	1-5 years	5	9.4	6	11.32	9	17	6	11.32	27	50.9	53	20.8	
	More than 5 years	17	23.95	9	12.7	15	21.1	9	12.7	21	29.6	71	27.8	
Duration of Admission	Less than 1 month	5	23.8	5	23.8	7	33.3	0	0	4	19.05	21	8.2	0.089
	1-6 months	49	25.1	22	11.3	32	16.4	33	16.9	59	30.3	195	76.5	
	More than 6 months	12	30.8	3	7.7	6	15.4	3	7.7	15	38.5	39	15.3	
Times of Admission	First Admission	57	33.3	24	14.04	24	14.0	24	14.04	42	24.6	171	67.1	0.000
	Frequent Admissions	9	10.7	6	7.14	21	25	12	14.3	36	42.9	84	32.9	
Accompa-nied persons	Absent	12	23.6	6	11.8	12	23.5	3	5.9	18	35.3	51	20	0.297
	Present	54	26.5	24	11.8	33	16.2	33	16.2	60	29.4	204	80	
Visitors	Absent	6	22.2	3	11.1	3	11.1	6	22.2	9	33.3	27	10.6	0.667
	Present	60	26.3	27	11.8	42	18.4	30	13.2	69	30.3	228	89.4	
Rehabilit-ation	Absent	6	20	0	0	9	30	6	20	9	30	30	11.8	0.083
	Present	60	26.7	30	13.3	36	16	30	13.3	69	30.7	225	88.2	
Walking aids	Absent	14	25.5	7	12.7	15	27.3	6	10.9	13	23.6	55	21.6	0.252
	Present	52	26	23	11.5	30	15	30	15	65	32.5	200	78.4	
Complicat-ion	Absent	24	22.2	18	16.7	24	22.2	21	19.4	21	19.4	108	42.4	0.001
	Present	42	28.6	12	8.2	21	14.3	15	10.2	57	38.8	147	57.6	
Co-morbidity	Absent	66	27.5	27	11.3	39	16.3	36	15	72	30	240	94.1	0.015
	Present	0	0	3	20	6	40	0	0	6	40	15	5.9	
Bedsores	Absent	45	28	23	14.3	23	14.3	20	12.4	50	31.1	161	63.1	0.147
	Present	21	22.3	7	7.45	22	23.4	16	17.02	28	29.8	94	36.9	
Total		66	25.9	30	11.8	45	17.65	36	14.1	78	30.6	255	100	

Discussion

The prevalence of depression was 74.1%, which is higher than many studies done across cultures. There was a strong correlation between degree of severity of depression and socio-demographic characteristics of the SCI inpatients. American meta-analysis (2014) of 19 studies found the mean prevalence estimate of depression diagnosis after SCI was 22.2% [22]. In a number of studies, it has been reported that depression scores vary between 20-40% in SCI patients [23,24]. Scivoletto et al in (1997) in Italian sample averaging 6 years post-SCI, found 16% reported significant symptoms of depression [25]. Migliorini et al. (2008) employed an Australian sample who averaged 19 years post-SCI, 37% were identified as depressed [26]. Dryden et al. (2004) study of 233 Albertans with SCI; 28.9% were treated for depression following their traumatic SCIs [27]. Bombardier et al. (2004) in a review found rates of major depression following SCI to vary widely across studies and can range from 7% to 31% of studied population [28]. Krause et al. (2008) surveyed 568 adult traumatic SCI inpatient rehabilitation clients; approximately 22% met self-reported symptoms consistent with major depressive disorder [29]. Bombardier et al. (2004) surveyed 849 SCI outpatients at one-year post injury and found 11.4% met criteria for MDD [27]. Krause et al. (2000) suggest a 42% overall rate of depression with a 21% probable rate of major depression [30].

Prevalence of depression of this study (74.1%) was lower than result of study done in Bangladesh (2007) on 167 spinal cord injured patients that found rate of depression to be around 80.24% [31]. Iranian study (2004) showed that the prevalence of depression in physically disabled veterans was (71%) [5], while recent Iranian study (2015) found 91 of 226 (40.2%) had moderate to severe depression [32]. Estimates of the prevalence of depression are affected by the nature of the measures used, how depression is defined, aging characteristics of the samples studied, and when symptoms are assessed post-injury.

Current study found that depression and severity of depression among spinal cord injured patients significantly associated with duration of disability; 66.4%, 90.6%, 76.1% for duration; 1 year, 1-5 years, <5 years respectively, while Richardson & Richards (2008), in a cross sectional study, found that rates of clinically significant depressive symptoms were reported by approximately 21%, 18%, 12% and 12% of SCI survivors surveyed at 1, 5, 15 and 25 years post injury, suggesting rates tended to decrease with time since injury [33]. Hoffman et al. (2008) followed 411 SCI model system participants and found approximately 20% of at 1-year post injury and 18% at year 5 post-injury reported symptoms consistent with major depression [34]. Pollard & Kennedy (2007) in a longitudinal analysis, found a substantial relationship between reported depressive symptoms at 3 months and approximately a decade post injury, with 38% and 35% of SCI survivors surveyed meeting a criterion for moderate depression at these times [35]. Kennedy & Rogers (2000) reported that anxiety, depression and hopelessness gradually increased beginning at week 30 post injury and continued until discharge from rehabilitation (week 48). At that point 60% of SCI clients scored above a clinical cut-off for depression (i.e. Beck Depression Inventory) [36]. This study showed significant gender association with depression and severity of depression, while Kalpakjian & Albright (2006) founded an absence of gender differences in probable major depression and symptom severity [37]. Turkish study (2014) found that depression was more frequent in females [6].

Current study found no statistical significance between depression and patient receiving rehabilitation or not. Krause et al. (2008) suggested that depressive symptoms may not peak during inpatient rehabilitation and it may take additional time for the “low point of emotional adaptation to appear” [38].

In this study, no any patient received treatment; dedication or psychotherapy. In a review of American veterans with spinal cord

injuries and disabilities, Smith et al. (2007) concluded that many may not be receiving adequate treatment for depression and the authors encouraged more aggressive screening and treatment [39]. Similarly, while a substantial percentage of their SCI clinic sample reported symptoms suggestive of major depression, Kemp & Krause (1999) found that none were receiving treatment (psychotherapy or medications) [40].

About 50% of causes of Spinal cord injury were bullets and shell explosions due to the security status and violence of Iraq and ongoing explosions and terrors events were major etiological factors associated with disability. Finding consistent with Sabah (2012) [18]. Violence is the commonest cause of Traumatic Spinal Cord Injuries in Iraq, which affect mainly the males at their most productive age.

The severity of depression of this study was the following; 25.9% mild depression, 11.8% moderate depression and 44.7% severe or very severe depression. Pakistanian study (2014) indicate the level of depression in people with physical disability, found that out of 35 individuals; 2.86% had mild mood disturbance, 2.86% had borderline clinical depression, 42.86% were moderately depressed, and 37.14% severely depressed and 14.29% were in extreme depression [41]. Robinson-Whelen et al. (2014) found that 41% of the women with spinal cord injury had depressive symptomatology in the mild to severe range. Nearly a third of the women had very sever depressive symptomatology [12].

In conclusions the prevalence of depression among SCI inpatients was 74.1%. About 45% of them were severely depressed. Severity of depression was significantly associated with sociodemographic characteristics, duration of disability, causes of injury, complications, and comorbidity. None of the depressed SCI patients received psychotherapy or medication. Violence was the commonest cause of Traumatic Spinal Cord Injuries. The results were compared with other studies from different cultures; prevalence of depressed spinal cord injured patients was higher than many studies and less than few.

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Authors Contribution:

Dr. Al Abbudi; consultant psychiatrist, data collection, data entry, data analysis, and writer of this paper. Dr. Ezzat, Social worker; data collection. Zebala, Psychologist, data collection. Hamdy, Psychologist, data collection. Al-Beedany, data collection and data entry. Farhan, Psychologist, data collection.

Conflict of interest

The authors declare no conflict of interest.

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Correspondence to Dr. Shalan J.R. Al-Abbudi
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The Role of ATG16L1 (Thr300Ala) Genetic Variants and Autophagy in Development of Acute and Chronic Urinary Tract Infection Caused by Uropathogenic *Escherichia coli*

Ghufran H. Abd[1] *MSc,* **Thanaa R. Abd Al-Rahman**[2] *PhD*, **Haider F. Ghazi**[3] *PhD*, **Haider S. Kadhim**[2] *PhD*

[1]1Alfarahidi University College, [2]Department of Medical Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Uropathogenic *Escherichia coli* (UPEC) is the major cause of urinary tract infection (UTI), establish quiescent intracellular bacterial reservoirs (QIRs). These latent reservoirs, which persist indefinitely, are resistance to antibiotic therapies and can induce recurrence. Autophagy related 16 like 1 gene (ATG16L1) Thr300Ala genetic variant confer an increased risk for the development of urinary tract infection caused by UPEC. This study aimed.

Objective To determine the possible relationship between the ATG16L1 Thr300Ala genetic variant and UPEC for the development of acute and chronic urinary tract infection.

Methods A total of 100 urine and blood samples were collected from patients complain from UTI, 20 blood samples of apparently healthy during the period between (November 2014 to May 2015) from two hospitals in Baghdad; Al-Imamein Al-Kadhimein Medical City and Al-Yarmauk Teaching Hospital. The age range exactly with mean ± SD or SE. *E. coli* were isolated by ordinary methods and the identification of non entero-pathogenic *E. coli* was performed at a group level by slide agglutination test with specific antisera. UPEC isolates were tested for their susceptibility to 12 antimicrobial agents by disc-diffusion method. ATG16L1 T300A genotyping was done by Species Specific Primer – Polymerase Chain Reaction (SSP-PCR), after genomic DNA extraction from each blood sample.

Results A total of sixty *E. coli* were isolated from 20 acute UTI (16 females and 4 males) and 40 chronic UTI (32 females and 8 males). There is a high rate of acute UTI among the age range exactly groups (≤10-20 years) and chronic UTI among age groups (21-40 years). Overall isolates had a complete resistance to Ampicillin and Gentamycin (100%), high resistance to Nalidixic acid (88%), Piperacillin (86%), Trimethoprime+Sulfa (84%), Cefotriaxon (80%), Ciprofloxacin (78%) and Cephalosporin (66%). Moderate resistance to Azithromycin (51%) and Cephalothin (50%) were seen. Whereas these isolates were highly susceptible to Imipenem and Nitrofuranton with the resistance rate 8% & 27% respectively. Ninety percent of the isolates were resistant to three or more antibiotics. The SSP-PCR result showed that a (89%) and (92%) of acute positive *E. coli* infection (P=0.009) and chronic positive *E. coli* infection (P=0.006) respectively were carried allele-G. While the occurrence of allele-G was (62%) in acute negative *E. coli* infection, and (30%) in chronic negative *E. coli* infection.

Conclusion There is a relationship between allelic variants of ATG16L1 gene with acute and chronic UTI. In the other hands, the risk of the present allele G was associated with increased susceptibility to infection by UPEC, which developing chronic and acute UTI.

Keywords *Escherichia coli*, autophagy, Thr300Ala genetic variant, PCR

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List of abbreviations: QIRs = Quiescent intracellular bacterial reservoirs, SSP-PCR = Sequence Specific Primer-Polymerase Chain Reaction, UPEC = Uropathogenic Escherichia coli, UTI = Urinary tract infection

Introduction

A urinary tract infection (UTI), one of the most common infection and difficult health problem in many different countries around the world [(1)]. Uropathogenic *Escherichia coli* (UPEC) is the major cause of UTI, establish quiescent intracellular bacterial reservoirs (QIRs). These latent reservoirs, which persist indefinitely, are resistance to antibiotic therapies and can induce recurrence [(2)].

Autophagy is a cellular degradation process that can eliminate intracellular pathogens by utilizing them to lysosomes for destruction [(3)]. In the other hands, ATG16L1 (autophagy-

related 16-like 1), its product plays an important role in the innate immune response and in the resistance to intracellular pathogens [4]. UPEC depend on a function of autophagy program [2].

The objective of this study was to determine the possible relationship between the ATG16L1 Thr300Ala genetic variant and UPEC for the development of acute and chronic urinary tract infection.

Methods

A total of 100 urine and blood samples were collected from patients suffering from UTI and 20 blood samples from apparently healthy controls. The differentiation between acute and chronic UTI according to clinical symptoms may be absent or include urinary frequency, urgency, dysuria, lower abdominal pain, and flank pain. Systemic symptoms and even sepsis may occur with kidney infection. Diagnosis of patients with UTI is based on analysis and culture of urine. Also, the diagnosis of control groups is depending on analysis and negative culture of urine so which considered as normal urinary tract(s) (apparently healthy control group) All urine samples were cultured on blood agar and MacConkey agar for identification of the bacteria. Api20E system was used for the final confirmation of the bacteria. For the identification of the bacteria at a group level, a slide agglutination test with specific antisera was done.

Antimicrobial susceptibility tests

Disk diffusion test was performed to determine the resistance patterns of isolated *E. coli* against twelve antibiotics includes Gentamicin (10 µg), Imipenem (10 µg), Ceftriaxone (30 µg), Ampicillin (10 µg), Nalidixic acid (30 µg), Ciprofloxacin (5 µg), Nitrofurantoin (50 µg), Trimethoprim + Sulfa (25 µg), Azithromycin (15 µg), Cephalothin (30 µg) and Piperacillin (30 µg).

DNA extraction

DNA was extracted from 300 µL peripheral blood EDTA containing tubes using DNA isolation kit (Wizard® Promega & QIAamp® Qiagen, USA). following manufacturer informations.

Sequence Specific Primer-Polymerase Chain Reaction (SSP-PCR)

Allelic discrimination was checked by SSP-PCR, for study groups using two sequences (Table 1). Three µl of extracted blood DNA was added to 12.5 µl GoTaq® Green Master Mix PCR master mix reaction with a 1.5 µl for each forward and reverse of two specific primers (Promega, USA) of ATG16L1 (Thr300Ala) in addition to internal control in a separated reaction mixture. The volume complete to 25 µl with a 1.5 µl ddH_2O. PCR products was 201bp for both allele A and allele G, allowing the discrimination of homozygous or heterozygous alleles. PCR program includes initial denaturation 94 °C for 3 min; denaturation 94 °C for 1 min, annealing 58 °C for 1 min, elongation 72 °C 1 min for 30 cycles the final extension at 72 °C for 10 min and hold at 4 °C using thermal cycler (Eppendroff-thermal cycler, Germany) and separated PCR-runs-for each allele.

Table 1. Sequence specific primers (SSP) and internal control primers (IC)

Gene	Primer	Sequence	Genomic position	Amplicon size (bp)
ATG16L1 (T300A)	F allele A	5´CCCCAGGACAATGTGGATA´3	2q37.1	201
	F allele G	5´CCCCAGGACAATGTGGATG´3		
	R**	5´AGGTGGAAAGGCTTGATATAAG´3		
β-globin	F	5`ACACAACTGTGTTCACTAGC´3	11p15.5	157
	R	5`GAAAATAGACCAATAGGCAG´3		

* F = Forward; ** R = Reverse

Gel-electrophoresis

PCR products were resolved in 1.5% agarose gel at 7 v/cm^2, 1 hr visualized by UV transilluminator and photographed by digital camera (Sony-Japan).

Statistical analysis

The statistical analysis performed with (SPSS) 19.0 and Microsoft Excel 2013.

Results

Patients and bacterial isolates

This study involved 100 Iraqi patients suffering from UTI, 34 with acute UTI and 66 with chronic – recurrent UTI in addition to 20 cases with normal urinary tract, which considered as apparently healthy group (HG). The age group ranging from ≤10 years to >50 years as shown in table (2).

Table 2. UTI patients and apparently healthy control were classified according to the age groups

Age groups		Study groups		Total
		Healthy	UTI	
≤10 years	Count	1	9	10
	%	5.0%	9.0%	8.3%
11-20 years	Count	3	19	22
	%	15.0%	19.0%	18.3%
21-30 years	Count	6	26	32
	%	30.0%	26.0%	26.7%
31-40 years	Count	5	24	29
	%	25.0%	24.0%	24.2%
41-50 years	Count	4	14	18
	%	20.0%	14.0%	15.0%
>50 years	Count	1	8	9
	%	5.0%	8.0%	7.5%
Total	Count	20	100	120
	%	100.0%	100.0%	100.0%
p value		0.948		

Classifications of UTI according to UPEC infection

There were 40 chronic UTI and 20 acute UTI showed positive bacteriuria, whereas 26 chronic and 14 acute UTI showed negative urine culture.

The occurrence of *E. coli* according to gender type

In UTI, there were 87/100 (87%) females and 13/100 (13%) males, while in apparently healthy control groups (HG) there were 11/20 (55%) female and 9/20 (45%) males. Sixteen out of twenty females (80%) and 4 out of 20 males (20%) were positive urine culture of *E. coli* in acute UTI, while 14/14 (100 %) female and there is no male were negative urine culture. In chronic – recurrent UTI with positive culture of *E. coli*, there were 32 out of 40 females (80%) and 8/40 males (20%). While in negative urine culture, there were 25/26 (96.15 %) females and 1/25 (3.85%) males.

Antibiotic resistance of Escherichia coli isolates

By the disc-diffusion method, *E. coli* isolates showed complete resistance to Ampicillin and Gentamycin (100%). High rate of resistance to

Nalidixic acid, Pepracillin, Trimethoprime + Sulfa, Ceftriaxone and Ciprofloxacin. Moderate to low resistance to Cephalosporin, Cephalothin, Azithromycin, Nitrofuranton and Imipenem as shown in figure (1).

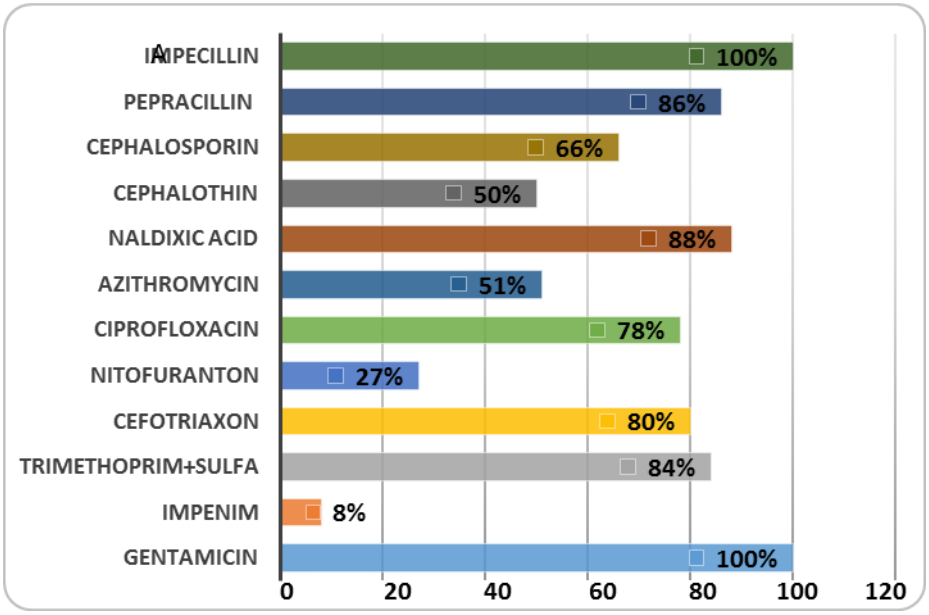

Figure 1. Antimicrobial susceptibility results of *Escherichia coli* isolates by the disc-diffusion methods

The association of ATG16L1 Thr300Ala genotypic variants with disease susceptibility

Most samples were investigated for the presence of rs2241880 ATG16L1 Thr300Ala polymorphism by SSP-PCR. This study showed that, higher percentages are of the homozygous mutant genotype (GG) 77.55% and 68.42%, and lower percentage for each heterozygous genotype (GA) 8.16% and 10.53% and homozygous wild type genotype (AA) 14.29% and 21.05% in chronic and acute UTI patients respectively. In the presence of allele G, the risk of incidence of acute and chronic UTI is more 10 times and 14.4 times than control respectively (Table 3).

The correlation between allelic frequency of rs2241880 ATG16L1 polymorphism and UPEC in UTI

Upon comparing the genotypic possibility (Ala300Ala, Thr300Ala and Thr300Thr) for 19 acute UTI and 49 chronic UTI, there were 14 acute UTI and 32 chronic UTI positive for urine culture with higher percentage of homozygous mutant genotype (GG) 85.71% and lower percentage of heterozygous genotype (GA) 7.14% and homozygous wild type genotype (AA) 7.14% in acute UTI. Simultaneously, there is a higher percentage in homozygous mutant genotype (GG) 87.50% and lower percentage of heterozygous genotype (GA) 9.38% and homozygous wild type genotype (AA) 3.13% in chronic UTI.

Regarding allelic frequencies, 25 out of 28 (89%) acute UTI were carrying allele G give *E. coli* positive compared with 3/10 (30%) of acute UTI negative for *E. coli*, whereas the percentage of chronic UTI carrying allele G with positive *E. coli* were 59/64 (92%) compared with 21/34 (62%) negative for *E. coli*. In the other hand, the risk of UPEC, which develop acute and chronic UTI was increased 19.44 times and 7.3 times respectively than those of *E. coli* negative (Table 4, Figures 2,3).

Table 3. Genotypic and allelic frequencies of rs2241880 ATG16L1 polymorphism in Iraqi acute, chronic UTI patients and apparently healthy controls

		Acute UTI	Chronic UTI	Healthy
ATG16L1 Genotype	**AA**	4 (21.05%)	7 (14.29%)	12 (70.59%)
	GA	2 (10.53%)	4 (8.16%)	1 (5.88%)
	GG	13 (68.42%)	38 (77.55%)	4 (23.53%)
	Total	19 (100.00%)	49 (100.00%)	17 (100%)
***p* value**	**vs healthy**	0.011	<0.001**	
ATG16L1 Allele	**G**	32 (80%)	84 (86%)	10 (29.0%)
	A	8 (20%)	14 (14%)	29 (71.0%)
	Total	40	98	34
Odd ratio (Confidence interval)	**vs healthy**	10 (2.114 to 43.60)	14.4 (3.866 to 53.64)	-
***p* value**	**vs healthy**	0.003*	<0.001**	-

*Statistical significant difference (p≤0.05), **= highly significant difference (p≤0.001)

Table 4. The association between the presence of uropathogenic *Escherichia coli* and ATG16L1 genotypic variants in UTI patients

		Acute UTI *E. coli*		Chronic UTI *E. coli*	
		Positive	**Negative**	**Positive**	**Negative**
ATG16L1 Genotype	**AA**	1 (7.14%)	3 (60.0%)	1 (3.13%)	6 (35.29%)
	AG	1 (7.14%)	1 (20.0%)	3 (9.38%)	1 (5.88%)
	GG	12 (85.71%)	1 (20.0%)	28 (87.5%)	10 (58.82%)
	Total	14 (100%)	5 (100%)	32 (100%)	17 (100%)
***p* value**	**vs no growth**	0.020*		0.009*	
ATG16L1 allele	**A**	3 (11.0%)	7 (70.0%)	5 (8.0%)	17 (38.0%)
	G	25 (89.0%)	3 (30.0%)	59 (92.0%)	21 (62.0%)
	Total	28	10	64	34
Odd ratio (Confidence interval)	**vs no growth**	19.44 (3.192 -118.5)	-	7.3 (2.323 -22.97)	-
***p* value**	**vs no growth**	0.009*	-	0.0006*	-

*Statistical significant difference (p≤0.05), **= highly significant difference (p≤0.001)

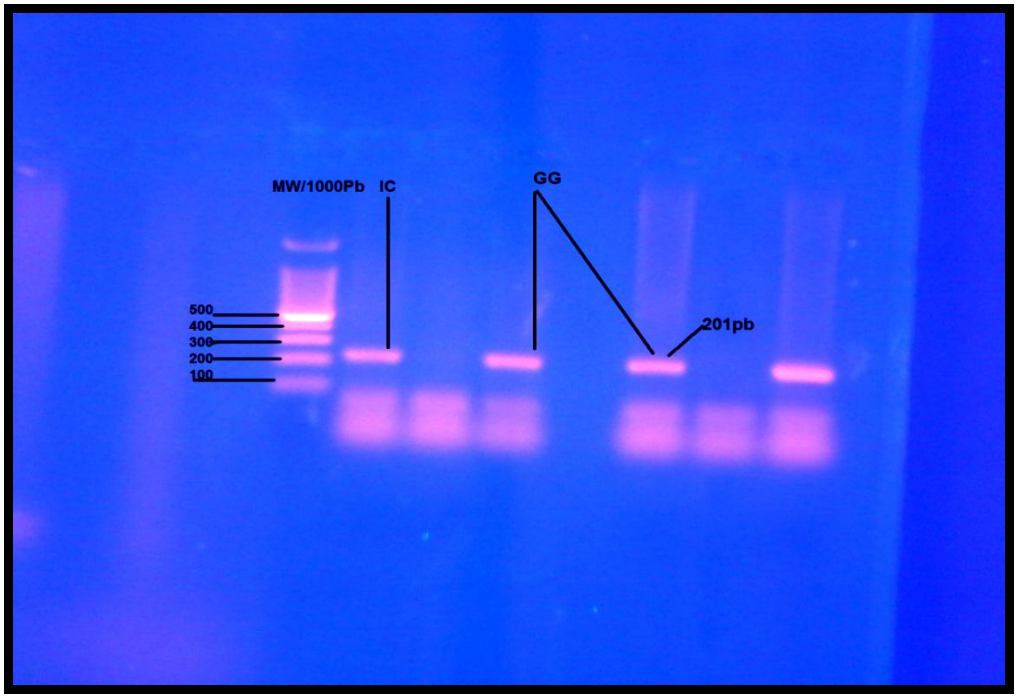

Figure 2. Electrophoretic profiles corresponding to homozyges mutant genotypes (GG) of the SNP T300A of the gene ATG16L1. M: molecular size standard (100 bp ladder), IC: Internal control (negative control or Housekeeping gene) with molecular size 157 bp and (GG)=201bp

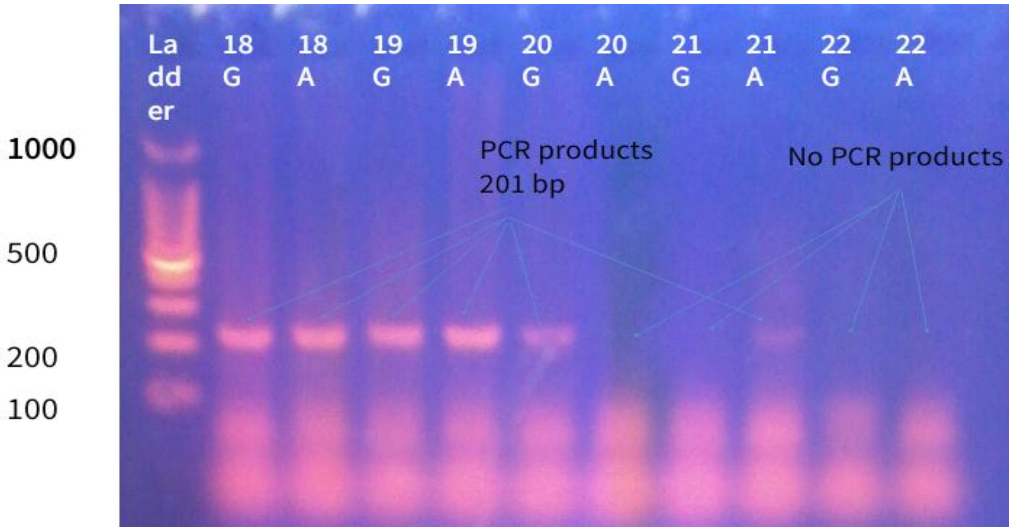

Figure 3. Electrophoretic profiles corresponding to PCR products, patients 20 homozyges mutant genotypes (GG). Patients 21 homozygous for allele A, patients 18 and 19 were heterozygous for allele A and G. patients, Molecular size standard (100 bp ladder, with molecular size of G-G=201bp

Discussion

Uropathogenic Escherichia coli (UPEC) cause 90% of UTI in human. These infections are highly prevalent in developing countries and are usually difficult to eradicate because these pathogens have acquired drug resistance [5,6].

In the current study, the prevalence of UTI with positive urine culture was in female (80%) more than in male (20%) in both acute and chronic UTI patients. The spread of infection was easy in females due to shorter urethra. This result was agreed with study conducted in Kurdistan region-Iraq that found 296 of UPEC strains were isolated from female accounting (81%) and male accounting (19%) [7].

This study showed that the incidence of infection increases with age and sexual activity. The rate significantly higher (45%) among 11-20 years in acute UTI in addition to 40 isolates from 66 patients with chronic UTI but less in age group 31-40 years and 41-50 years that gave *E. coli* positive. The National Center for Health Statistics reported that up to 20% of young females with acute cystitis develop recurrent UTI's, this due to the fact that postmenopausal women may undergo bladder or uterine prolapse, loss of estrogen that causes a change in the vaginal flora or loss of lactobacilli, which results in periurethral colonization with gram-negative aerobes (*E. coli*) [8].

This result also observed that 90% of the *E. coli* isolates are very high percentage of resistance was noted against Ampicillin and Gentamycin (100%) followed by Nalidixic acid (88%), Pepracillin (86%), Trimethoprime + Sulfa (84%), Cefotriaxon (80%) and Ciprofloxacin (78%). The current study was agreed with other studies in Iraq [9] and India [10] when they found that very high resistance to trimethoprim Sulfmethaxazol (94.4 %) and Nalidixic acid (92.6%) of *E. coli* in UTI patients respectively.

This study found that the Imipenem and Nitrofuranton were to be the most potent antimicrobial agents with the resistance rate (8%) and (27%) respectively and this was agreed with researches reported find same susceptibility to Imipenem and Ampicillin [7].

This study also conducted to investigate the association of ATG16L1 Thr300Ala genotypic variants. To the best of our knowledge, it is believed that this study is the first study in Iraq concerning the risk of autophagy related gene 16 like 1 protein T300A SNP in urinary tract infection. The current results showed that genotype GG was more prevalent among patients suffering from acute and chronic UTI patients followed by genotypes AA when compared with apparently healthy controls. While other study concluded reported that, the genotype AG was more prevalent among patients and controls, followed by genotypes AA and GG in Crohn's disease [11].

The PCR results showed that the frequency of the allele G polymorphism T300A was higher in the group of patients with acute and chronic UTI (80% & 86% respectively). This leads to the fact that increases susceptibility of acute and chronic urinary tract infections conferred by polymorphism T300A. The results of healthy controls strongly suggest that the active autophagy process was present in the normal human superficial urothelial cells, are the target cells for UPEC invasion and Atg16L1protein expression. In this study, there was also a predominance of the allele A (wild type) among healthy controls (71%). These results agreed with previous study describing the active autophagy process in normal colonic mucosa [12].

The present study found the homozygous mutant genotype GG was more prevalent among acute and chronic UTI patients with positive *E. coli* compared with negative *E. coli*, whereas lower percentage frequencies of (GA) and (AA) from chronic and acute UTI with positive *E. coli*.

In other hand, the risk factor of susceptibility to infection by UPEC was increased on 19.44 times in chronic UTI and 7.3 times in acute UTI on the presence of allele G that made protection to persistence *E. coli* as intracellular bacterial communities. Early invasion and colonization of the bladder were not dramatically altered by the Atg16L1 deficiency-induced urothelial ultrastructural changes. Strikingly, however, in the presence of these

abnormalities, UPEC appears less able to reside in the intracellular niches to persist in the urotheliumas QIRs [11].

The Atg16L1-deficient may elevate proinflammatory cytokine levels this may have had beneficial effect to promote rapid elimination of bacteria, which may prevent additional bacterial invasion into underlying urothelial cells and result in faster clearance of bacterial load leading to restoration of a normal urothelium [13]. While Other study showed that, the invasion of UPEC into epithelial cells was inhibiting proinflammatory cytokine production [14].

In conclusion, there is a relationship between allelic variants of ATG16L1 gene with acute and chronic UTI. In the other hands the risk of the present allele G was associated with increased susceptibility to infection by UPEC, which developing acute and chronic UTI.

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Authors Contribution:

Dr. Kadhim supervise this paper as part from a thesis. Abd and Dr. Abd Al-Rahman prepared, performed and did the tests and sampling. Dr. Ghazi and Dr. Abd Al-Rahman interpreted the results of the research.

Conflict of interest

The authors declare no conflict of interest.

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Correspondence to Dr. Thanaa R. Abdulrahman
E-mail: thanaraaa1970@yahoo.com
Thanaa.rasheed@colmed-alnahrain.edu.iq
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Detection of *Listeria monocytogenes* in Placenta of Aborted Women

Khalid W. Qassim[1] *MSc,* **Azhar A.F. AL Attraqchi**[2] *PhD*, **Yaarub I. Khatab** *FIBMS (Path)*

[1]Department of Pathology and Forensic Medicine, [2]Dept. of Medical Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background *Listeria monocytogenes* (*L. monocytogenes*) is a Gram-positive, facultative intracellular bacterial pathogen that can cause a severe invasive disease (listeriosis), mainly in immunocompromised, elderly individuals, and pregnant women, characterized by sepsis, neonates and miscarriage.

Objective To evaluate the association of listeria monocytogenes in abortion in a group of Iraqi women.

Methods A cross-sectional study was designed and included 250 placenta tissues obtained from aborted women, each placenta sample was divided into two parts each about 50 gm in weight, one stored in 10% formaldehyde solution to use for histopathological study using Hematoxylin and Eosin (H&E) staining procedure, while the second part cut into small pieces about 15 gm and washed and stored in 5 ml normal saline solution 0.85% to use in detection of the presence of the bacterium using conventional bacteriological methods.

Results Out of 425 placenta samples only 250 were diagnosed to be placentitis and only 15 isolates of *L. monocytogenes* were isolated and constitutes 6% of the total number of placentitis. Distribution of placentitis due to listeria and age groups have been shown no statistical significant differences (P = 0.099). Also, there was no statistical significance difference between the percentage of isolated *L. monocytogenes* and the time of gestation in aborted women with placentitis (P value was 0.689), and also no significance in association between number of abortion and isolation of *L. monocytogenes* (P = 0.689).

Conclusion No association between *L. monocytogenes* and recurrent abortion or with time of gestation or the age of the patients.

Keywords *Listeria monocytogenes*, placenta, aborted women

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List of abbreviations: *L. monocytogenes = Listeria monocytogenes*

Introduction

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive, non-spore-forming, motile, facultative anaerobic, rod-shaped intracellular bacterial pathogen that can cause a severe invasive disease (listeriosis), mainly in immunocompromised, elderly individuals, and pregnant women, characterized by sepsis, meningitis and miscarriage [(1)].

Listeriosis during pregnancy may lead to intrauterine infection, which may result in severe complications like preterm labor, spontaneous abortion, and stillbirth and/or infection of the neonate which may result in high morbidity and mortality rates [(2)].

Despite its clinical importance, a little is known about the molecular and cellular mechanism leading to placento-fetal infection, or the role

of pregnancy in the development of listeriosis. One explanation for the increased susceptibility to listeriosis during pregnancy is the immunological conditions of mammalian reproduction, where the maternal immune system tolerates paternal alloantigens expressed in fetal tissues. Since then, pregnancy has been regarded as a state of immunosuppression; in particular, of the cell-mediated arm of the immune system [3].

A decrease in cell-mediated immunity might explain the increased susceptibility to infection with the bacterial pathogen, *L. monocytogenes* and this is the reason for increased incidence of listeriosis during pregnancy. Infections of human with *L. monocytogenes* has been traced to contaminated foods [4,5]. Once ingested, *L. monocytogenes* is able to cross the intestinal barrier; invasive disease is usually occurred secondary to hematogenous dissemination and typically leads to infection of the placento-fetal unit during pregnancy or to meningitis in immunocompromised patients [6].

This study aimed to evaluate the association of listeria monocytogenes in abortion in a group of Iraqi women.

Methods

A cross-sectional study was designed that included 250 placenta tissues obtained from aborted women attended Al-Imamein Al-Kadhimein Teaching Hospital in Baghdad during the period from June 2014 to November 2015.

Preparation of the samples

Each placenta sample was divided into two parts, each about 50 gm in weight, one stored in 10% formaldehyde solution to use for histopathological study, while the second part cut into small pieces about 15 gm and washed and stored in 5 ml normal slain solution 0.85% to use in bacteriological study.

Preparation of Formalin-Fixed, Paraffin-Embedded tissues (FFPE)

Placental tissue s were sectioned into 3 mm slices and transferred into formalin (10%); fixative volume was 20 times that of tissue on a weight per volume, tissue s were fixed for a minimum 48 hours at room temperature then processed, using gentle agitation, as follows: 70% ethanol for 2 h, 80% ethanol for 2 h, 90% ethanol for 2 hours, absolute ethanol for 2 hours, absolute ethanol for 2 h, xylene for 2 h, xylene for 2 h, first paraffin at 58 °C for 2 h, second paraffin at 58 °C 2 h.

Embedding tissues in paraffin blocks

Small amount of molten paraffin was put in mold, then transfer tissue into mold, placing cut side down, as it was placed in the cassette. Then the cassette was filled with paraffin and left to completely cooled and hardened, from each block, one section of 5µm thickness was taken and stained with Hematoxylin and Eosin for the histopathological diagnosis.

Isolation of *L. monocytogenes*

Placental samples were cuts into small pieces about 15-20 gm and then washed using normal saline solution 0.85%, then put in sterile tubes containing 5 ml normal saline solution 0.85%, tube contents were homogenized by mixing them thoroughly for 10 min to release the bacteria to the solution. Then, 0.5 ml of the placental suspension was inoculated in a sterile tube containing 10 ml of brain heart infusion broth and incubated at 37 °C for 24 h, then 0.5 ml of the bacterial growth was then inoculated on blood agar, nutrient agar, and PALCAM agar and incubated at 37 °C for 24 h.

Laboratory diagnosis

The isolation and identification of *L. monocytogenes* were performed according to Collee et al. 1996 and McFaddin, 2000 [7,8].

Statistical analysis

The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft Excel 2013. Numerical data were described as mean and standard error. Independent t-test used for comparison between groups. While, categorical data described as count and percentage, Chi-square test used to estimate the association between variables. The lower

level of accepted statistical significant difference is P value below to 0.05.

Results

Out of two hundred and fifty placental samples only fifteen isolates of *L. monocytogenes* were isolated and identified, the total number of the positive *L. monocytogenes* samples constitutes only 6% of the total number of examined placental tissues, the distribution of the isolates according to the age groups of the aborted women revealed that the age group (25-29) years represented the highest number concerning *L. monocytogenes*, which was 12 (80%), while the age group (30-34) years constituted the smallest number concerning this bacteria, which was 3 (20%), (Table 1), however no statistical significant differences between patients' age groups concerning *L. monocytogenes*, in which P value was (0.099). Distribution of samples according to the time of gestation showed that the highest percentage of *L. monocytogenes* was 9 isolates (60%), which were isolated from aborted women in the first trimester while 5 isolates were collected from those in the second trimester (33.3%) and only 1 listeria isolate was isolated from aborted women in the third trimester (6.7%).

Table 1. The distribution of placentitis due to Listeria monocytogenes according to the age groups

Age group (years)	Number of samples	Number of *L. monocytogenes* isolates	Percentage
20-24	33	0	0.0
25-29	127	12	80.0
30-34	75	3	20.0
35-39	15	0	0.0
Total	250	15	6.0
P value		0.099	

Table 2 shows that there is no statistical significant difference between the percentage of isolated *L. monocytogenes* and the time of gestation, in which P value was (0.689), however, the distribution of samples according to the number of abortion was unevenly, in which 12 isolates were isolated from women with a first abortion while only 3 isolates from those with recurrent abortions.

Table 2. The distribution of *L. monocytogenes* isolates according to the time of abortion

Trimester	Number of samples	Number of *L. monocytogenes* isolates	Percentage
First	171	9	60.0
Second	70	5	33.3
Third	9	1	6.7
Total	250	15	6.0
P value		0.689	

Table 3 shows that the highest percentage of listeria was associated with the first abortion was (80%), while those with recurrent abortion showed only (20%) for the isolation of the bacterium. The results have shown no significant association between number of abortion and isolation of *L. monocytogenes*, (P = 0.869). All obtained placenta samples were examined histopathologicaly and according to the reports of the pathologist. Out of 425 placenta samples only 250 were diagnosed to be placentitis and were adopted for this study. (Figure 1) and (Figure 2).

Table 3. The distribution of *L. monocytogenes* isolates according to the number of abortions

Number of abortions	First abortion	Recurrent abortions	Total
Number of samples	204	46	250
Number of *L. monocytogenes* isolates	12	3	15
Percentage of total listeria isolates	80%	20%	6%
P value	0.869		

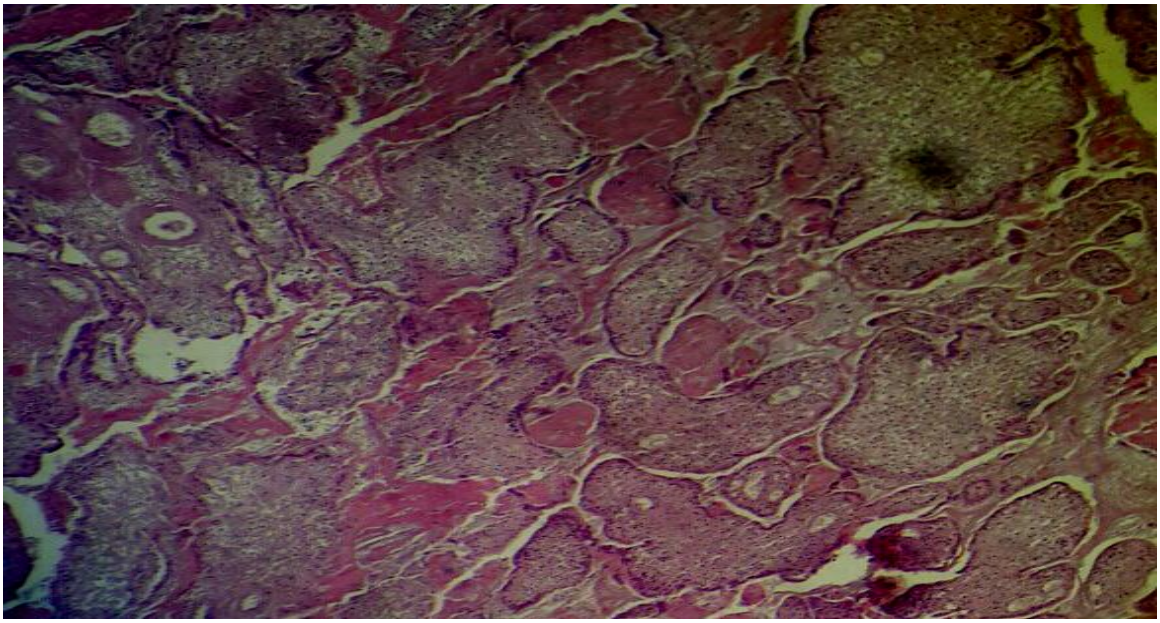

Figure 1. Placenta section showing evidence of placentitis with dense heavy mixed inflammatory cells included both PMN and mature lymphocytes infiltrate intravillous and stromal core (X10).

Discussion

Pregnant women are more susceptible to *L. monocytogenes* infection than healthy individuals; *L. monocytogenes* invades the placenta and result in preterm labor and fetal death [9,10]. During pregnancy, *L. monocytogenes* infection can be asymptomatic or may give rise to subclinical symptoms such as a nonspecific fever, however the development of placento-fetal infection may result in abortion, stillbirth or disseminated neonatal infections, notably granulomatosis infantiseptica [11,12].

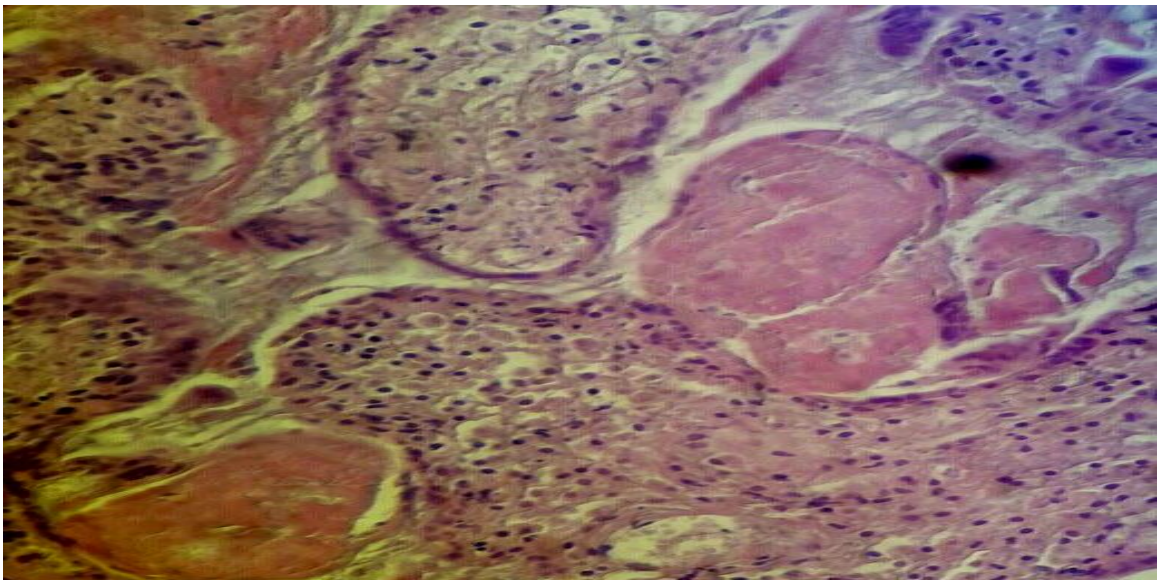

Figure 2. Placenta section showing evidence of placentitis with dense heavy mixed inflammatory cells included both PMN and mature lymphocytes infiltrate intravillous and stromal core (X40).

Jackson et al. in 2010 mentioned that the percentage of *L. monocytogenes* isolated from placenta of pregnant women was 1.6% [(14)]. While Al-Shukri in 2011 found that only five isolates of *L. monocytogenes* out of three hundred three vaginal swaps, where responsible for abortion [(15)]. However, the variation in *L. monocytogenes* percentage might be due to sample size, which was 425 placenta samples, cultivation and identification methods; this study used bacteriological methods while others used molecular methods like nucleic acid assay kits, polymerase chain reaction (PCR) which is rapid but with high cost. Furthermore, the abortion associated bleeding may acts as a cleaning factor that prevents the *L. monocytogenes* colonization [(16)], so we washed the placenta tissue which contains the bacteria to collect them. On the other hand, the cytotoxic T-lymphocytes that found in the placenta tissue were able to recognize and kill the *L. monocytogenes* infected cells by apoptosis, which may lead to the damage of the placental tissue and lead to fetus death [(17)] and this may affect the result because our samples are placenta tissues.

Distribution of the isolates according to the age groups of the aborted women showed that the age group of (25-29) years was the highest among those with listeriosis where the percentage was (80%), while the age group (30-34) years was the lowest where the percentage was (20%), these results agree with Listeria Annual Summary, (2010) which states that the median age of pregnancy-associated cases is 28 years [(18)], but the results disagree with Tahery et al. in 2009 who found the most cases of listeria has been seen in the age group of (41-46) (36%), followed by age groups of (36-40) (27%), (31-35) (13%) and (26-30) [(19)]. These differences in results may due to variation in life style, socioeconomic status; methods of diagnosis, ethnic differences etc…. However, the results of this study have been shown no statistically significances among different age groups and the rate of *L. monocytogenes*, this is agreed with Jamshidi et al. 2009 who mentioned that the seropositivity for *L. monocytogenes* was not age dependent [(20)].

L. monocytogenes was unevenly distributed regarding the time of gestation; however, most isolates were isolated from aborted women at

the first trimester, in which represents the highest percentage of isolation, on the other hand, only one listeria case isolated from aborted women in the third trimester. However, result of the current study disagreed with most of others, most studies showed that the isolation of *L. monocytogenes* during pregnancy is strongly associated with abortion occurred at the third trimester. Listeriosis occurs mainly in the third trimester and this may be due to deficient cell mediated immunity but some cases have been observed at earlier gestational ages, the incidence at lower gestational ages may be underestimated due to reluctance to culture of aborted fetal tissue or products of conception [21], because the doctors don't send samples to the laboratory, however this variation in isolation of the bacteria and time of gestation might be due to the treatment regimen followed by Iraqi gynecologist, which permit antibiotics treatment in wide range during third trimester and since *L. monocytogenes* infection during pregnancy might be asymptomatic or flu-like symptoms so it may miss diagnosed, so listeria infection will be excluded.

Most of listeria cases were associated with the first abortion and only three cases of listeriosis were occurred in those in recurrent abortion. The results also showed that there were no statistical significant differences in association between isolation of Listeria monocytogenes and the number of abortion. Some studies have suggested an association between chronic carriage of *L. monocytogenes* and recurrent abortion, but this suggestion has not been approved [22].

However, negative culture results do not exclude the presence of an infectious organism. *L. monocytogenes* may be difficult to identify in cultures because of bacterial overgrowth and in areas in which multiple organisms may be present [23].

The diagnosis of listeria infection during pregnancy is difficult because most cases are asymptomatic and may lead to abortion and stillbirths furthermore, the continuous failure to isolate the bacterium in blood and tissue cultures, those reasons have been reinforcing the importance of placental histopathological examination to confirm the clinical suspicions [24].

The placenta histological examination showed diffused scattered, tiny, lesions consisting of villous microabscesses with foci of necrosis and peripheral palisaded histiocytes. Focal villitis have been seen with the presence of neutrophils between the trophoblast and the villous stroma.

There were dense heavy mixed inflammatory cells included both polymorphnuclear and mature lymphocytes infiltrate of intravillous stromal core which is a general feature associated with most cases of placentitis, histological findings typical of *L. monocytogenes* infection [25].

In conclusion, this study found that *L. monocytogenes* is associated with some cases of abortion and that the rate of isolation of listeria monocytogenes is not associated with female age, time of gestation or number of abortions.

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Authors Contribution:

Qassim collected the cases, performed the bacterial tests and analyzed the results. Dr. AL Attraqchi helped in the study design and supervising the work. Dr. Khatab participated collection of cases and performed the histopathological examination.

Conflict of interest

The authors declare no conflict of interest.

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Correspondence to Dr. Azhar A.F. AL Attraqchi
E-mail: tariq_963@yahoo.com
dr.azhar.ibrahim@colmed-alnahrain.edu.iq
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CO_2 Diode Laser Hemorrhoidectomy: Clinical Experience with 150 Patients

Ibrahim F. Noori *CABS, FICS*

Dept. of Surgery, College of Medicine, Basra University, Iraq

Abstract

Background Hemorrhoids are the most common benign anorectal conditions. About half of population are affected by hemorrhoids during their life time. Conventional surgical hemorrhoidectomy, which was the standard treatment for many decades are associated with remarkable and intolerable pain that may last up to 3 to 6 weeks, which makes more patients reluctant to surgery. Laser hemorrhoidectomy is relatively new promising modality with less postoperative pain and less complications with fast wound healing and early return to work.

Objective To assess the validity, feasibility and the outcomes of using the CO_2 diode laser for treatment of symptomatic hemorrhoids.

Methods A prospective study conducted for the period from September 2013 to April 2015, included 150 patients (135 male and 15 female), with symptomatic first, second and third degree piles with age ranged from 24 to 83 years (mean 48.7 year) submitted to CO_2 diode laser hemorrhoidectomy with either the coagulation mode or cutting mode of 30 Watt diode laser surgical machine. The procedures were done under local anesthesia as a day case surgery by single surgeon.

Results The results showed that the operative time ranges from 15 to 30 minutes (mean 23.5 minutes). Postoperative pain scores by visual analog scale (VAS) in the first, second and third postoperative days were 2.9, 2.1, 1.8 respectively. The pain decreased rapidly after the first week and reached to zero after 14 days postoperatively. The complications rate recorded in this work was 7.3%. All these complications were mild and can be dealt with conservatively; redo surgery was not required in any case. All patients were discharged 2 to 4 hours postoperatively and were followed for 3 to 6 months.

Conclusion Hemorrhoidectomy by CO_2 diode laser is effective, and very quick outpatient procedure with very mild postoperative pain and low or negligible rate of complications. It associated with rapid healing and fast recovery that most patient can return to normal daily activity within 2 to 5 days. These results are considered a big advantage upon conventional hemorrhoidectomy.

Keywords Hemorrhoids, laser hemorrhoidectomy, CO_2 diode laser

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List of abbreviations: None.

Introduction

Hemorrhoids or piles are the commonest anorectal diseases. They affected about 50% to 70% of population by the ages of fifty to sixty-five years [1]. The disease tends to runs in family and affect male slightly more than female. Although hemorrhoidal cushions are normal vascular structures found in anal canal above the dentate line that aid in fine continence and found along the final distribution of terminal branches of superior rectal artery at 3, 7, and 11 o'clock in a lithotomy position, they are infrequently referred to them until they are enlarged and

dilated, then the term "hemorrhoid" is described as a pathologic process [1,2].

Clinical presentations of hemorrhoids range from being completely asymptomatic, bleeding (1st degree), mucosal prolapse that return spontaneously or manually after defecation (2nd and 3rd degree piles respectively) and permanent prolapsed with itching and pruritis (4th degree). Treatment is usually required for all symptomatic hemorrhoid to remove the hemorrhoid and relieve the symptoms.

The pathogenesis of hemorrhoid is not completely understood. Several theories were put to explain the development of hemorrhoid. The arterial flow or hypervascularization theory adapted by Aigner et al [1] is one of these theories. They suggest that the three terminal branches of superior rectal artery have large diameters, greater blood flow, in addition to a higher peak velocity and acceleration velocity in patients with hemorrhoidal disease when compared to those of healthy individuals. According to this theory, the arterial overflow in the superior hemorrhoidal arteries will lead to dilatation and enlargement of hemorrhoidal vascular plexus [3].

The conventional excisional hemorrhoidectomy whether open (Milligan-Morgan) or closed (Ferguson) is usually associated with remarkable postoperative pain which may last up to 2 to 3 months in addition to other complications like bleeding, urine retention, infection, anal stenosis and could be recurrence [3,4].

Laser hemorrhoidectomy by CO_2 diode laser is new ambulatory outpatient procedure for treatment of selected patient with 1st, 2nd and 3rd degree piles in which the hemorrhoidal arterial flow feeding the enlarged vascular plexus is stopped by laser vaporization, coagulation and by excision [4]. The utilization of laser for removal of hemorrhoid in selected patients are expected to have many advantages over the classical surgical hemorrhoidectomy such as less postoperative pain with smooth and comfortable postoperative course, no hospitalization is required with faster return to work.

The aim of this study was to present and evaluate our initial clinical experience with 150 consecutive patients presented with symptomatic hemorrhoid of first, second and third degree submitted to ambulatory hemorrhoidectomy using the CO_2 diode laser.

Methods

This is a prospective study conducted for the period from September 2013 to April 2015 in which, 150 consecutive patients with symptomatic first, second and third-degree piles were selected to have hemorrhoidectomy using CO_2 diode laser. They were 135 male and 15 female patients with age ranges between 24 and 83 years (mean 48.7 years). Patients with American Society of Anesthesiologist (ASA) grade III and IV, previous anal surgery fourth degree piles, prolapsed, strangulated and thrombosed piles, recurrent piles, and piles with concomitant anorectal diseases were excluded from laser hemorrhoidectomy and offered for conventional surgical excision by open Milligan-Morgan procedure.

A detailed history was obtained and through physical examination were offered to all patients in this study. Routine preoperative investigations were done, which include complete blood count, blood sugar, blood urea, hepatitis viral profile and electrocardiography for all patients above 40 years. Proctoscopy was done for all patients to exclude secondary hemorrhoid or to exclude other pathology of bleeding per rectum.

All operations were done as outpatient ambulatory procedures. Bowel preparations by rectal enema was not required. Patients before procedures were pre-medicated by receiving 75 mg diclofenac and/or tramadol intramuscular injection. The procedure started with the patients lying in lithotomy position. The perianal area is infiltrated by injection of about 20 ml mixture containing equal amount of 2% lidocaine with adrenaline and 0.5% bupivacaine with 5 ml Na-bicarbonate to reduce the acidic effect of anesthetic mixture.

The laser machine used in these procedures was 30-Watt diode laser surgical machine, model IB 411 made by Innobri Technology company (Figure 1). Patients were informed about all details of the procedure and informed verbal consent was obtained from each patient prior to operation. All operations were conducted by the same single surgeon.

Figure 1. 30 W DIODE laser surgical machine

Procedure

- The procedure starts with anal dilatation with aid of lidocaine jell 5% to relax the anal internal sphincter and to bring the internal hemorrhoid in to the view.
- The CO_2 diode laser machine setting is adjusted on 10-Watt, time on 45 ms, time off 30 ms.
- Having the laser cable of 400 micron on the hand piece, a cerebrospinal fluid needle (CSF) is inserted on the hand piece that the laser optic fiber pass through and only 1-2 mm appeared from the needle tip.
- C-shaped anoscope lubricated with 2% lidocaine jell introduced inside the anal canal.
- Starting with a large pile which is usually at 3 o'clock position, an Allis forceps is applied at muco-cutaneous junction of the piles and pull the piles outward and downward to bring it outside the anal canal, using the laser in a cutting setting mode, the pile is excised with a pinpoint accuracy. Stitches are not needed as the laser seals the blood vessels as it cut so. The risk of incontinence due to internal sphincter injury is absent because the laser beam is so small and there is very clear view of hemorrhoid.
- After removal of large pile, smaller piles are dealt with now. With anoscope still inside, the pile is grasped at its tip with an Allis forceps, the CSF needle with the laser fiber optic tip (1-2 mm bare fiber out) as the laser setting on coagulation mode, the needle got inside the pile from the outer part down up to the pile root submucosally to avoid the perforation of the pile from inside to prevent bleeding.
- The CO_2 diode laser stars to coagulate the vascular pedicle (root) of the pile by 5 to 10 shots generated at a power of 10 Watt causing shrinkage of the pile mass. The coagulation depth reaches to the depth of about 5 to 10 mm. the rest of the pile is further coagulated by passing the laser fiber tip into all parts of the pile. It is not needed to make more than one entry spot to the hemorrhoid, it needs only one entry at the top of the pile and from it we can do laser effect on the entire of the pile. It takes about 3 minutes for each pile to be fully coagulated.

- The same process was repeated with the rest of the piles.
- Mixed pile (has internal and external components) are dealt with by separation of both components by a bridge of anal skin. The internal pile then will be coagulated and vaporized with a coagulation laser beam and the external part excised with a cutting mode laser.
- At the end of the procedure, sterile gauze dressing soaked with lidocaine jell is applied externally over the anal verge. The time taken by this procedure ranged between 15 minutes to 25 minutes with mean time of 21.6 minutes (Figure 2).

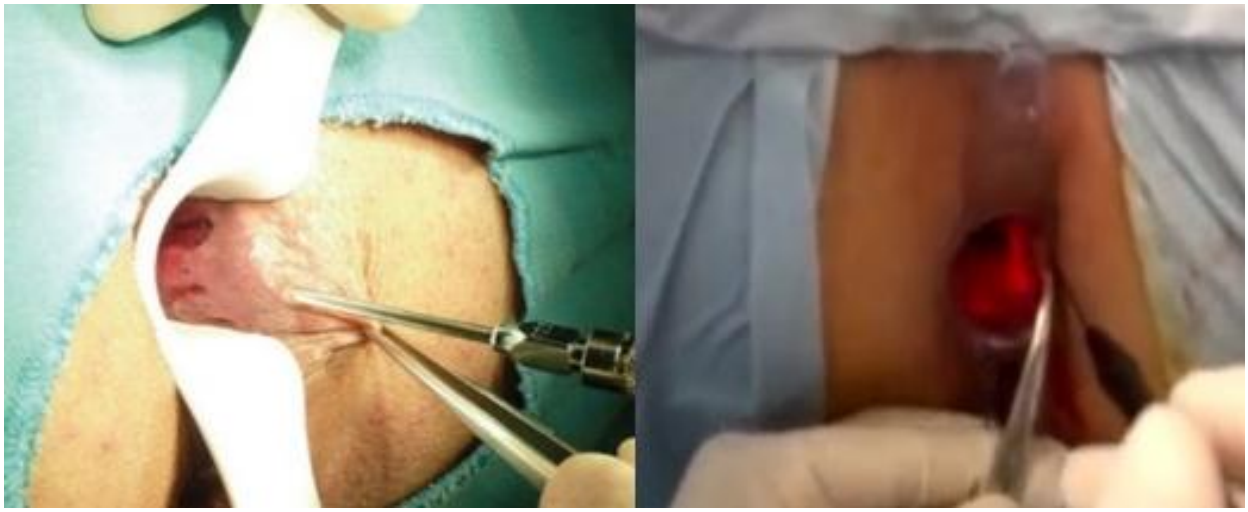

Figure 2. Laser hemorrhoidectomy procedure

After surgery

After the procedure, the majority of the patients has a good feeling of wellbeing. There was no pain as long as the long acting anesthesia (0.5% bupivacaine) still active and working (Figure 3).

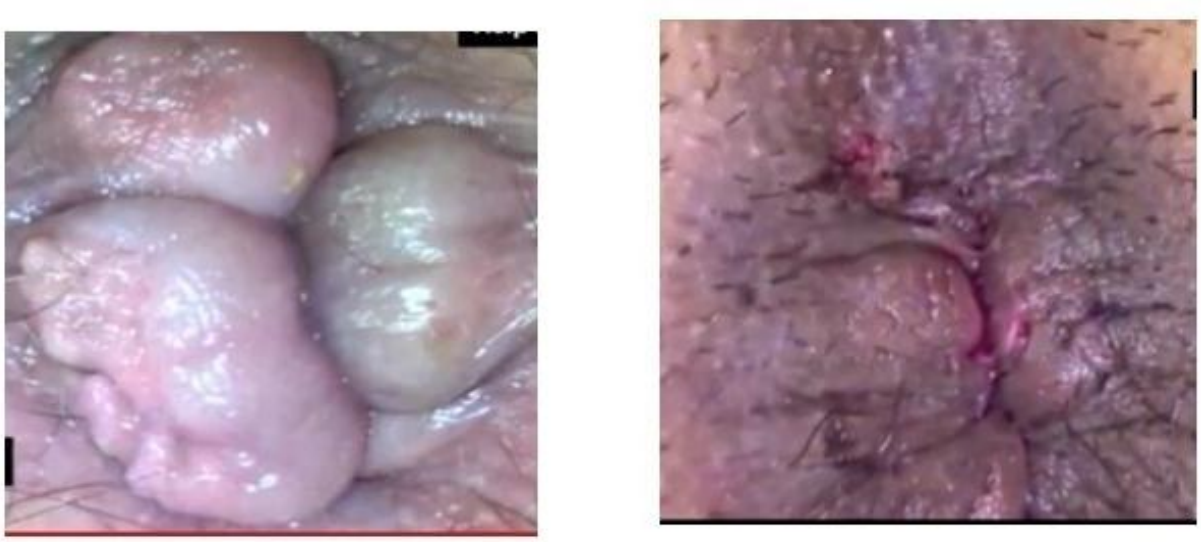

Figure 3. Preoperative and immediate postoperative laser hemorrhoidectomy

All patients were send home 2 to 4 hours postoperatively. Drugs prescribed for patients to be taken at home include pain killer in form of ibubrufen and Tylenol or Panadol

(acetaminophene) tablets, stool softener like bisacodyl (dulcolax) tablet, and antibiotics like metronidazole and cefepime tablet. Injectable medications were rarely required.

Patients were informed prior to discharge that any discomfort or pain felt at home can be greatly alleviated by sitting in a warm water (sitz path) 2 to 3 times daily. Patients were ordered to be seen on fifth postoperative day or earlier when they have any questions or complain.

Postoperative pain, complications, healing rate, recurrence rate and patient satisfaction with results of operation were recorded and assessed. Postoperative pain was evaluated by using the visual analog scale in which 0 represent no pain and 10 score represents the worst and intolerable pain. The fellow up period in this study was 3 to 6months.

Results

Laser hemorrhoidectomy by CO_2 diode laser machine was carried out on 150 consecutive patients (135 male and 15 female) with mean age 48.7 years range from 24 to 83 years. The age distribution of patients included in this study is shown in table (1).

Table 1. Age distribution of patients presented in the study

Age	Sex		Total
	Male	Female	
20-39	32	3	35
40-59	77	6	83
≥60	26	6	32

They presented with symptomatic first, second and third-degree piles with no or mild permanent mucosal prolapsed (Table 2). None of the patient was incontinent preoperatively (Wexner score 0-2).

Operative time ranges from 15 to 30 minutes, mean 23.5 minutes. Hospital stay was 2 to 4 hours. All patients were discharged after pack checking to ensure no bleeding and almost with no pain or very mild pain that can be dealt with oral or parentral analgesics. Postoperative pain, complications, wound healing, patients' satisfaction with the procedure and recurrence rate were all documented and evaluated. The main patients' symptoms were bleeding and prolapse of the piles masses as shown in table (2).

Table 2. Distribution of symptoms among patients

Symptoms	No	%
Bleeding without prolapsed (1st degree)	16	10.7
Bleeding with prolapsed during defection only (2nd degree)	62	41.3
Bleeding with permanent prolapsed (3rd degree)	72	48

Postoperative pain as assessed using the visual analog scale (VAS) is demonstrated by table (3). The pain score in the first, second, and third postoperative day were 2.9, 2.1 and 1.8 respectively. The pain score will decrease gradually over the first week to reach 0.9 in the seven-postoperative day. Most patients (no. 136, 90.7%) record pain score of 0 (no pain) after 14 days postoperatively.

The overall complications rate among patients in this study was 7.3% (11 patients) (Table 4). Bleeding was the most common immediate and early post-operative complication, developed in 5 patients (3.3%). It was mild

bleeding and stopped conservatively and no re-intervention was required. All cases were dealt with successfully by dressing with absorbable hemostatic gelatin sponge.

Three patients in this study developed wound infection. These infections were managed by intravenous antibiotics in form of metronidazole and ceftriaxone for 5 days and all patients were improved and healed.

Urine retention occurred in 2 (1.3%) elderly male patients. Only one patient required catheterization due to concomitant benign prostatic hypertrophy and the other 2 patients were treated conservatively by sedation, fluid restriction and warm path.

Anal stenosis observed only in one patient, it was managed by anal dilatation under local anesthesia together with application of 0.2% GTN cream for 7 days.

All patients treated by laser hemorrhoidectomy in this study were able to return to their normal activities within 5 to 7 days and complete healing was achieved and accomplished in all patients after 14 to 21 days postoperatively.

Anal incontinence was not detected in any patient probably due to pin point accuracy and precision of laser beam used in these procedures. Furthermore, recurrence was not recorded in any patient throughout the 6 months fellow up period.

Table 3. Pain score by VAS among patients by Laser Hemorrhoidectomy

VAS	Day 0	Day 1	Day 3	Day 7	Day 14	Day 21
0-2	35/150	65/150	115/150	140/150	150/150	150/150
2-5	112/150	83/150	33/150	9/150	0/150	0/150
>5	3/150	2/150	2/150	1/150	0/150	0/150

Table 4. Postoperative complications among patients by laser hemorrhoidectomy

Complications	No	%
Bleeding	5	3.3
Urine retention	2	1.3
Wound infection	3	2
Delay wound healing	1	0.6
Anal stenosis	1	0.6
Incontinence	0	0
Recurrence	0	0
Overall complications	11	7.3

Patients satisfaction about the procedures was also studied (Table 5). Majority of patients included in this study (no. 137, 91.3%) were very satisfied with the procedure and its outcomes. They preferred laser hemorrhoidectomy over conventional surgical hemorrhoidectomy because of less postoperative pain, speeder recovery and fewer complications.

Table 5. Patients satisfaction with laser hemorrhoidectomy

Patients	Operation results				Total
	Satisfied		Unsatisfied		
	Very satisfied	Satisfied	Fair	Unsatisfied	
Male	59	67	7	2	135
Female	65	2	2	2	15
Total	65	72	9	4	150

Discussion

The majority of patients with piles were reluctant to surgery due to postoperative pain, which may lead to delay of surgical treatment. Traditional excision and suture ligation hemorrhoidectomy whether by open method (Milligan-Morgan) or by closed method (Ferguson) is still most commonly practiced symptomatic and complicated hemorrhoids [2]. Postoperative pain is the most common and disabling complication after these procedures due to highly sensitive anoderm. The pain could last for 3 to 6 weeks. Other less frequent complications of these operations include bleeding, anal stricture and recurrence. Therefore, uneventful and relatively painless procedure are the main aims of both the surgeon and the patient [4].

Postoperative pain after excisional hemorrhoidectomy may results from cutting the somatic nerves in highly sensitive anoderm, sphincter spasm, mucosal damage, insertion of hemostatic gauze or could be due to sutures ligation of piles pedicle [2,5].

Laser hemorrhoidectomy is a new treatment modality employing laser energy to coagulate, vaporize, disintegrate and excise hemorrhoid [2,5]. It is relatively painless procedure when compared with excisional or stapled hemorrhoidectomy. Early reported results were promising and good [4-6]. The main principle and justification of hemorrhoidal procedure is based upon the fact tans-anal hemorrhoidal de-arterializations that by reducing the inflow of the arterial blood causes a gradual and progressive reduction of hemorrhoidal mass volume, which in turn results in progressive improvement of hemorrhoidal related symptoms. Besides, the pinpoint laser beam can be used to excise the prolapsed and external piles with high accuracy and precision [5,6].

Laser hemorrhoidectomy is a clinical procedure when hemorrhoids are disintegrated and removed using specialized equipment that emits laser. It is similar to other procedures that are performed to manage hemorrhoids [7]. The ultimate goal of laser hemorrhoidectomy is to get rid of the piles completely, relieve the patients' symptoms, postoperative pain and less complications with prompt healing and recovery. It is an outpatient or office-based procedure, which is very quick to perform and needs no hospitalization [6,8].

Laser was first introduced and used in anorectal surgery in the 1960s by Senagore et al. [9] who used Nd:YAG laser in their series of 86 patients. They concluded that there are no advantages with the use of Nd:YAG laser for hemorrhoidectomy upon traditional excisional hemorrhoidectomy. Later on, the results have been greatly improved with the advent of CO_2 diode laser together with the development of scanned and pulsed laser. Plapler et al. [10] recorded in their study of 350 patients who submitted to hemorrhoidectomy with CO_2 diode laser that laser hemorrhoidal operations resulted in less postoperative pain and better outcomes when compared with conventional hemorrhoidectomy.

The results of this study showed that the treatment of hemorrhoid with CO_2 diode laser results in much low score of postoperative pain. The pain score didn't exceed 3 degrees score on visual analog scale in the first 3 postoperative day and decreased gradually to

reach to the base line 2 weeks after intervention. Patients in this study needed mild analgesics in form of NSAIDs and warm sitz path to manage their postoperative pain and narcotic analgesics were not required or used for any patient. Zahir et al. [11] reported in their study of 50 patients with second and third-degree piles who submitted to hemorrhoidectomy by CO_2 diode laser that the procedure has better postoperative course and remarkably less pain. Wang et al. [12] in their study, which compared the outcomes of conventional excisional hemorrhoidectomy with CO_2 diode laser hemorrhoidectomy showed that 11% in laser hemorrhoidectomy group needed analgesia versus 56% in conventional hemorrhoidectomy.

A reduction in postoperative pain and discomfort after laser hemorrhoidectomy is mainly due to sealing the superficial nerve endings, minor tissue damage, and faster wound healing.

The complications rate in this study was 7.3%. All these complications were minor. No patient had suffered a major postoperative complication that requiring re-intervention or hospitalization. Bleeding was most common complication. It was light bleeding, never cause alarm and no more than three days after intervention. It stopped conservatively and blood transfusion never required. Other less frequent complications such as wound infection (2%), urine retention (1.3%) and anal stenosis (0.6%) were managed conservatively and there was no need for further surgery. Giamundo et al. [13] recorded in their study that laser hemorrhoidectomy procedure as less painful and more effective than rubber band ligation. They found that laser hemorrhoidectomy associated with fast recovery and low complications rate.

All laser hemorrhoidectomy procedures were done as a day case surgery. All patients were discharged 2 to 4 hours after procedure. Hospital admission was not needed, for any patient.

A study of 750 patients presented with second and third-degree hemorrhoids conducted by Hodgson et al. [14] who submitted to laser hemorrhoidectomy, 98% successful rate was recorded and patients' satisfaction was 99%. The current study reported a satisfaction rate of 94.7%. Another study conducted at university of Sao Paolo in Brazil [15] found that CO_2 diode laser hemorrhoidectomy is superior to conventional hemorrhoidectomy by being hemostatic, less postoperative pain and complications, fast healing, less damage to the tissues and neighboring structures, and bactericidal as well. They also showed that 94% of patients required no or simple postoperative analgesics, 14% of patients need narcotics. The incidence of postoperative complications like bleeding and stenosis were about 1%.

A study by Maloku et al. [16] observed in their study of 40 patients that there were statistically significant differences between laser hemorrhoidplasty and open surgical hemorrhoidectomy inoperative time and early postoperative pain and they concluded that laser hemorrhoidectomy was more effective than open surgical hemorrhoidectomy. Similar retrospective comparative study of 1024 patients with third and fourth degree hemorrhoids by Saad et al. [17] who randomly subjected to either surgical excisional hemorrhoidectomy or CO_2 diode laser hemorrhoidectomy, they found that CO_2 diode laser hemorrhoidectomy was safe and easy procedure with lower rate of complications, shorter hospital stay and cost effective. These findings were also recorded by Awazli [18] who used 10600 nm CO_2 diode laser for 25 patients complaining from symptomatic hemorrhoids for many years.

It worthwhile to mention that the laser is inherently therapeutic, sealing off nerves therefore, patients treated by laser hemorrhoidectomy have a minimum postoperative discomfort. Furthermore, laser results in closure of small blood vessels with small invisible light and thus it facilitates the surgeon to operate in blood less white field.

Laser hemorrhoidectomy procedure is commonly performed under local anesthesia with sedation as a day case surgery and thus the chance of any major complications from this procedure are rare.

This study concluded that CO_2 diode Laser hemorrhoidectomy is an effective procedure characterize by decreased tissue damage, minimal blood loss and less postoperative pain and postoperative complications. It is associated with short operative time and performed as a day case surgery and hospitalization never required. It results in fast recovery and earl return to normal daily activity. It is safe, feasible procedure that commonly performed under local anesthesia. It require a well-trained and expert surgeon and expensive machine (CO_2 diode laser system machine).

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Evaluation of Antemortem and Postmortem Levels of Organochlorine Pesticides in a Sample of Iraqi People

Harb S. Hmood[1] *MSc,* **Noor M. Ali**[1] *PhD*, **Muataz A. Al-Qazzaz**[2] *PhD*

[1]Dept. of Chemistry and Biochemistry, [2]Dept. of Pathology and Forensic Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Organochlorine pesticides have long been widely used in agriculture and in public health as highly effective pest control agents. They are lipophilic and have prolonged half-lives of years to decades; as a consequence, they accumulate in human adipose tissues and can cause chronic toxicity after long-term exposure.

Objective To detect and measure the concentrations of organochlorine pesticides (trans-nonachlor and oxychlordane) in postmortem organs and fatty tissue as well as their concentrations in antemortem serum and fatty tissue samples and study their correlation with lipids in order to reveal the need for human monitoring.

Methods The study was conducted on 40 antemortem samples of blood and fatty tissues and 41 postmortem samples of blood and different organ tissues to determine their lipid concentrations and detect metabolites of organochlorine pesticides and asses their correlations using spectrophotometer and HPLC techniques.

Results The study observed that there was normal serum concentration of triglyceride (TG) and elevated cholesterol level, which were verse correlated with elevated serum concentrations of trans-nonachlore and oxychlordane pesticides. Serum concentrations of TG were (153.75 mg/dl) within "normal" range while mean serum of total cholesterol was (209.89 mg/dl) elevated above normal range. Percentage of concentration of serum to lipid trans-nonachlordane was (40.28 mg/dl) higher than that of oxychlordane was (28.42 mg/dl) in living subjects. The study observed that elevated concentrations of trans-nonachlor more than oxychlordane in postmortem tissue organs.

Conclusion The study revealed that traces of organochlorines (trans-nonchlore and oxychlordane) were detected in human serum, fatty tissue and postmortem organs and positively correlated with some lipid profiles indicating the presence of human contamination. Both trans-nonchlore and oxychlordane were higher in lipid tissue than in serum and other tissues among postmortem cases.

Keywords Organochlorine, trans-nonachlore, oxychlordane, postmortem, lipid profile

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List of abbreviations: None

Introduction

Pesticides defined as any substance or mixture of materials proposed for destroying, preventing, modifying and resisting somewhat pest, or substances administered to animals for the controlling insects, arachnids or other pests [(1)]. A pesticide may be a biological agent, disinfectant, antimicrobial, chemical substance or device

used to combat any pest [1]. The major classes of pesticides include:

1. Fungicides - used against fungi.
2. Herbicides - used against weeds.
3. Rodenticides - used against rodents.
4. Bacteriocides - used against bacteria.
5. Molluscides - used against mollusk.
6. Nematocides - used against nematodes.
7. Algicides - used against algae.
8. Insecticides - used against insect [1].

Organochlorine pesticides are classes of hydrocarbon compounds characterized by their cyclic structure, number and location of chlorine atoms and low volatility. They were widely used in agriculture and for pest control after they were introduced in the 1940s [2].

Organochlorine components have low levels in the environment. They are formed naturally. Many of their uses have been invalid or restricted since their ecological persistence and potential adverse special effects on natural surroundings and social health [3].

Organochlorine composites are lipophilic and expected to be bio accumulated in human body and to be found in human adipose tissue, breast milk and blood. The levels of organochlorine substances generally are almost the same at various body tissues but less in the blood [4]. Measurable levels of organochlorine pesticides have been originated in human fatty tissues, plasma and breast milk through the world [5]. Chlordane, a human-made chemical mixture structurally similar to organochlorines, was widely used on agricultural crops and as a germicide in United States until 1988. Chlordane consists of > 140 isomers; the most abundant include trans-chlordane, cis-chlordane, trans-nonachlor, beta-chlordane, and heptachlor [6].

In humans, the predominant chlordane-related contaminants detected are trans-nonachlor and oxychlordane (major metabolites of the chlordane). Chlordane's have a 10-20 years half-life in soil [6]. Chlordane block inhibitory neurotransmitters and result in central nervous system toxicity and when acute high doses are taken seizures and paralysis occur [7]. Chlordane perhaps carcinogenic to humans [8]. A significant trend for Non-Hodgkin's Lymphoma (NHL) was noted for in-creased levels of α-chlordane residues in dust [9]. One study had reported a significantly increased risk of rectal cancer for "ever use" of chlordane [9]. McGlynn et al. in 2008 found significant associations between risk of testicular germ cell tumors and serum levels of cis-nonachlor, trans-nonachlor, and total chlordanes. Similar analysis for seminoma revealed significant associations between seminoma and levels of cis-nonachlor, trans-nonachlor, oxychlordane, and to-tal chlordanes [10].

The objectives of this study were to detect and measure the concentrations of organochlorine pesticides (trans-nonachlor, and oxychlordane) in postmortem organs and fatty tissue as well as their concentrations in antemortem serum and fatty tissue samples and study their correlation with lipids in order to reveal the need for human monitoring.

Methods

A cross sectional study was conducted on 40 living individuals (antemortem group) and 41 autopsy cases (postmortem group) to study organochlorines.

Antemortem cases

Forty patients were included in the study after gotten their written consents. They were undergoing surgical operations in Al-Imamein Al-Kadhimein Medical City. Ten ml of Blood and 5-10 g of fatty tissue samples were collected from each patient and stored in the freezer at 4 °C for blood samples and -8 °C for fatty tissue samples. Each sample was sent for chemical analysis at the Department of Chemistry and Biochemistry Laboratory, College of Medicine, Al-Naharian University immediately to determine lipid profile and to measure the level of organochlorine pesticide regardless of their age and sex. Patients with chronic disease were excluded from the study. Blood samples were separated by 3000 rpm fast centrifugation for 10 minutes and the serum was collected and divided into two equal parts, first part for lipid

profile test and other part for levels measurement of organochlorine pesticides.

Organochlorine pesticides test in antemortem cases

Four hundred µl of serum were added to 50 µl of 25% sulfosalicylic acid to deproteinization serum and centrifugation in a centrifuge for 10 minutes, which were separated from the supernatant and leaved the precipitate that contain proteins, and added the same amount of ethanol, which to offset protein to a 1:1 ratio to make sure full protein shift (deproteinization). Finally, 20 µl was taken from prepared sample and injected to high performance liquid chromatography (HPLC).

Serum lipid profile test of antemortem cases

Procedure of measure of cholesterol and triglyceride (TG) in antemortem samples

Procedure for TG

Lipoprotein lipase hydrolysis TG to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphate, which oxidized by the enzyme glycerol phosphate oxidase to form hydrogen peroxidase. The hydrogen peroxidase further reacts with phenolic compound and 4 aminoantipyrine by the catalytic action of peroxidase to form a red colored quinonemine dye complex. Intensity of the color formed is directly proportional to the amount of TG present in the sample.

Triglycerides lipoprotein lipase $\xrightarrow{lipoprotein\ lipase}$ Glycerol + Free Fatty Acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol 3 Phosphate + ADP

Glycerol 3 Phosphate + O_2 $\xrightarrow{Glycerol\ 3\ PO}$ Dihydroxyacetone phos. + H_2O_2

H_2O_2+ 4 Aminoantipyrine + Phenol $\xrightarrow{Peroxidase}$ Red Quinoneimine dye + H_2O

Addition Sequence	B (ml)	S (ml)	T (ml)
Working Reagent	1.0	1.0	1.0
Distilled Water	0.01	--	--
Triglycerides Standard (S)	--	0.01	--
Sample	--	--	0.01

Mix well and incubate at 37 °C for 5 minutes or at room temperature (25 °C) for 15 minutes. Measure the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) against the Blank within 60 minutes at wave length 505 nm.

Triglycerides in mg/dl = $\frac{Abs.\ S}{Abs.\ T}$ x 200.

Procedure for cholesterol

This method for the measurement of total cholesterol [1,2] in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample.

Cholesteryl esters $\xrightarrow{CE}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{CO}$ Cholestenone + H_2O_2

4-AA + Phenol $\frac{H2O2}{POD}$ Quinoneimine + 4H2O

Procedure

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes.
3. Mix and incubate the tubes 10 minutes at room temperature or 5 minutes at 37 °C.
4. Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

Total cholesterol mg/dl = $\frac{A\ sample}{A\ standard}$ x C Standard

Tubes	Blank	Sample	Calibration standard
R1. Monoreagent	1 mL	1 mL	1 mL
Sample	--	10 µL	--
Calibration Standard	--	--	10 µL

Postmortem cases

Sampling of postmortem cases

On the other hand, samples from 41 fresh and healthy autopsy cases at the Medicolegal Directory in Baghdad were included in this study during the period. These samples were weighted (5-10 g) from (liver, brain, kidney and adipose tissue) absolute in alcohol and send for chemical analysis in the same day. These samples were taken from death victims due to traumatic causes. Decomposed bodies and those with chronic diseases was also excluded from the study. All cases were matched according to their sex, age and region of residence.

Extraction of tissue

The method of chemical extraction for isolation and analysis of brain, liver, kidney and adipose tissue samples. Briefly, one gram of tissue samples was taken one ml of n-hexane was added to dissolve tissue for homogenies solution by homogenizer and add another one ml of n-hexane for operation again and add one ml of the same solution to become for 3:1 and continues until became a homogeneous solution, which then centrifuged at (3000 rpm)/min for 10 minutes, then the supernatant was taken and added it (50 µL) of 15% 5-Sulfo-salicylic acid for deproteinization and then separated by centrifuged again for another 10 minutes at (3000 rpm)/min, took the supernatant and add the same amount of ethanol with a 1:1 ratio to make sure a sample deproteinization clear again with package. After centrifugation, separated into two layers, the upper class was taken from the sample and injected a HPLC technique [11,12].

Measured concentration in total lipid

Total lipid content was quantified gravimetrically in adipose tissue using a previously reported method. The lipid adjusted concentration of the pesticide obtained by dividing the measured pesticide residue concentration in the total tissue sample by the decimal fraction of the sample that consisted of ether-extractable lipid. The total lipid content of each specimen was estimated from its total cholesterol & triglycerides levels by using a summation method. Analytical results for OC pesticides were reported on a lipid-adjusted basis (nanograms per gram or parts per billion). The lipid-adjusted concentration of an analyte was given by:

$$C \text{ lipid adjusted} = [CONC/TL] \times 102.6$$

Where CONC is the concentration of an analyte in a sample as weight per gram of sample:

TL (total lipid) = (2.27 × total cholesterol mg/dL + triglycerides + 62.3)

Both wet-and lipid-basis adipose tissue serum ratios were calculated by dividing each adipose tissue concentration by each serum concentration.

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Results

Antemortem group

Forty patients were engaged in the study. Females were 23 constituting 57.5% while males were 17 that were 42.5%. The mean age was 37±11.31 years.

Mean serum concentrations of triglycerides was within normal ranges while mean serum of the total cholesterol was elevated above normal range as listed in table (1).

The means for trans-nonachlor in serum and in tissue were higher than that for oxychlordane.

There were significant differences between their concentrations in serum and tissue as listed in table (2) and shown in figure (1).

Table 1. Mean, standard deviation and range of age and lipid parameters in antemortem group

Parameters	Mean±D	Range
Age (years)	37±11.31	20-65
Serum Triglyceride (mg/dl)	153.75±41.71	72-358
Serum Cholesterol (mg/dl)	209.89±26.11	162.8-268.3
Total lipid (mg/dl)	692.49±74.54	571.96-857.34

Table 2. Comparison of serum and tissues trans-nonachlor and oxychlordane in antemortem group

Parameters	Serum mean±SD	Tissue mean±SD	*P* value
Transnonachlor (ng/g)	1.65±0.19	4.37±0.35	< 0.001
Oxychlordane (ng/g)	0.78±0.51	1.13±0.09	< 0.001

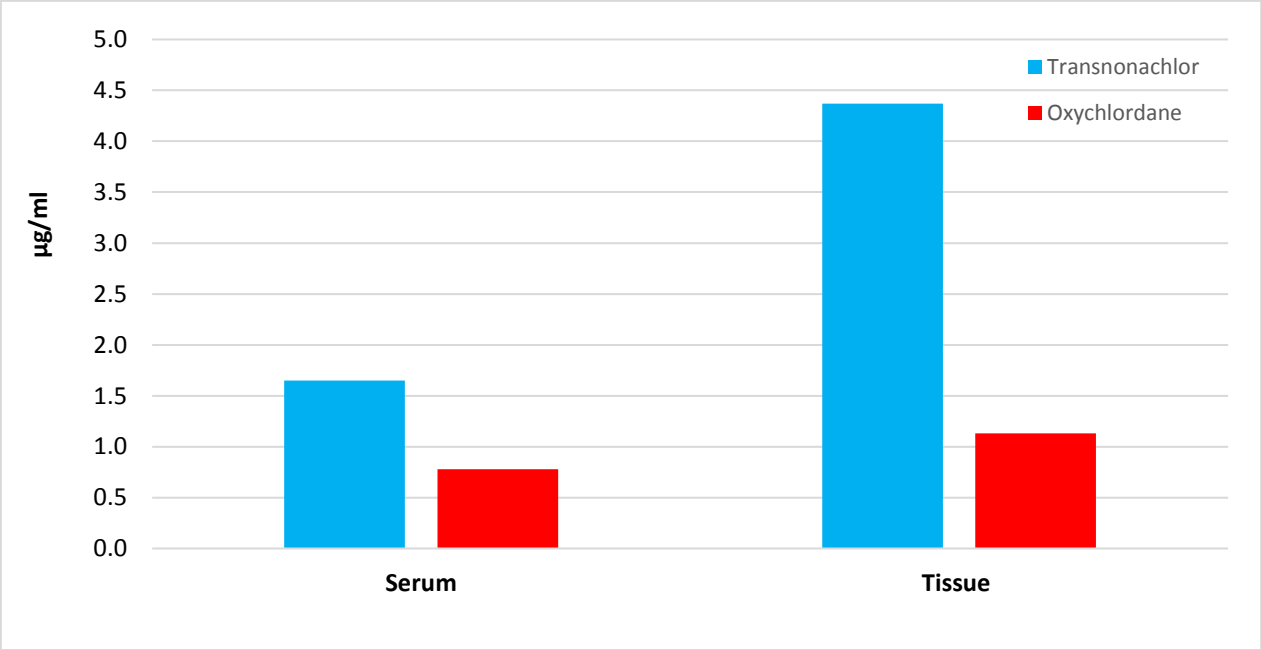

Figure 1. Serum and tissues trans-nonchlor and oxychlordane in antemortem group

Percentage of concentration of serum to lipid trans-chlordane was (40.28±7.3) higher than that of oxychlordane 28.42±11.74 as listed in table (3).

There were no significant differences between male and female regarding different compounds in the study as shown in table (4). The correlation coefficient between parameters were non-significant values as listed table (5).

There were significant positive and negative correlation between serum concentrations of trans-nonchlor and oxychlordane and their serum triglyceride and cholesterol as listed in table (6).

Table 3. Percentage of serum to lipid organochlorines

Percentage of lipid to serum	Mean	Range
Trans-nonachlor	40.28±7.3	29.48-60.89
Oxychlordane	28.42±11.74	8.24-49.14

Table 4. Comparison of parameters between females and males in antemortem group

Parameters	Females (n=23) mean±SD	Males (n=17) mean±SD	*P* value
Age (yrs.)	37.04±10.86	36.94±12.23	0.978
Triglyceride (mg/dL)	156.14±34.93	150.51±50.44	0.695
Cholesterol (mg/dL)	212.43±24.98	206.44±27.97	0.488
Serum Trans-nonachlor (ng/g)	1.67±0.16	1.61±0.22	0.348
Serum Oxychlordane (ng/g)	0.77±0.52	0.8±0.52	0.847
Tissue Trans-nonachlor (ng/g)	4.4±0.27	4.34±0.44	0.645
Tissue Oxychlordane (ng/g)	1.12±0.09	1.14+0.09	0.549
Total lipid	700.67±73.03	681.43±77.35	0.431
% of conc. of serum to lipid (Trans)	39.36±7.2	41.52±7.47	0.364
% of conc. of serum to lipid (oxy)	27.99±10.93	28.99±13.07	0.800

Table 5. Correlation of age with other parameters in antemortem group

Parameters	r	*P*
Serum Triglyceride	-0.085	0.603
Serum cholesterol	0.170	0.296
Serum Transnonachlor (ng/g)	0.009	0.955
Serum Oxychlordane (ng/g)	0.101	0.535
Tissue Transnonachlor (ng/g)	0.178	0.272
Tissue Oxychlordane (ng/g)	-0.047	0.773
lipid equation	0.087	0.592
% of conc. Of serum to lipid (Trans)	0.001	0.994
% of conc. Of serum to lipid (Oxy)	-0.134	0.410

Table 6. Correlation of serum triglyceride and serum cholesterol with serum and tissue trans-nonchlor and oxychlordane in antemortem group

Parameters	Serum TG		Serum Chol.	
	r	*P*	r	*P*
Serum Transnonachlor (ng/g)	-0.063	0.699	0.039	0.810
Serum Oxychlordane (ng/g)	0.126	0.440	0.128	0.430
Tissue Transnonachlor (ng/g)	-0.296	0.063	-0.069	0.673
Tissue Oxychlordane (ng/g)	-0.183	0.258	-0.028	0.863

Postmortem Group

Forty-one postmortem cases were involved in the study. They included healthy victims died from traumatic injuries. Decomposed bodies were excluded from the study. Females were only 7 (%17) while males were 34 (%83). There were no significant values between male and female regarding organochlorines pesticides (trans-nonchlor, oxychlordane) in different organs and tissues as listed in table (7).

There were highly significant differences between different organs and tissues regarding trans-nonachlor concentration as listed in table (8).

There were highly significant differences between different organs and tissues regarding Oxychlordane concentration as listed in table (9) and as shown in figure (2).

The correlation coefficient values of age with other parameters were non- significant and only positive correlation with kidney oxychlordane and in tissue for these, two organochlorine as listed table (10).

Table 7. Comparison of parameters between females and males postmortem group

Parameters	Females (n=7) mean±SD	Males (n=34) mean±SD	*P* value
Age (years)	52.14±14.54	39.38±13.66	0.064
Kidney Trans-nonachlor (ng/g)	2.24±0.28	2.28±0.27	0.745
Kidney Oxychlordane (ng/g)	1.33±0.09	1.38±0.2	0.304
Liver Trans-nonachlor (ng/g)	3.69±0.45	3.62±0.33	0.717
Liver Oxychlordane (ng/g)	1.93±0.28	1.94±0.14	0.862
Brain Trans-nonachlor (ng/g)	4.37±0.26	4.56±0.25	0.128
Brain Oxychlordane (ng/g)	2.42±0.19	2.39±0.18	0.700
Tissue Trans-nonachlor (ng/g)	6.14±0.28	6.09±0.31	0.681
Tissue Oxychlordane (ng/g)	2.57±0.17	2.58±0.22	0.904

Table 8. Comparison of different tissues trans-nonachlor in postmortem group by ANOVA

Type of tissue	mean±SD	*P* value
Kidney	2.27±0.27	< 0.001
Liver	3.63±0.35	
Brain	4.52±0.26	
Tissue	6.1±0.3	

Table 9. Comparison of different tissues oxychlordane in postmortem group by ANOVA

Type of tissue	mean±SD	*P* value
Kidney	1.37±0.19	< 0.001
Liver	1.94±0.17	
Brain	2.4±0.18	
Tissue	2.57±0.21	

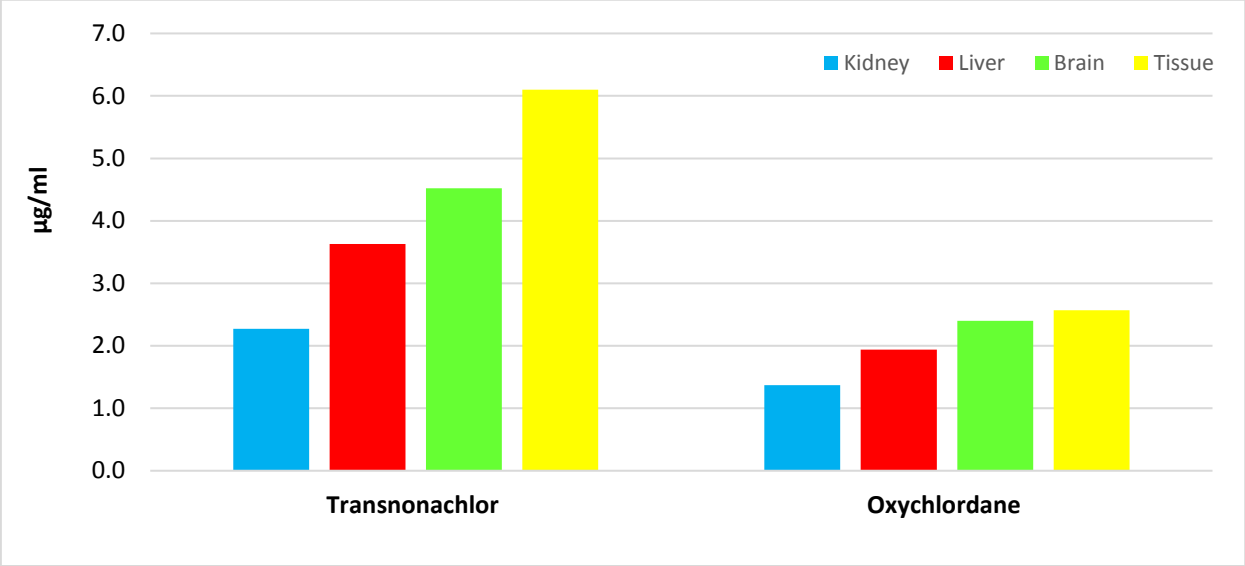

Figure 2. Different tissues trans-nonchlor and oxychlordane in Postmortem group

Table 10. Correlation of age with other parameters in postmortem group

Parameters	r	*P*
Kidney Trans-nonachlor (ng/g)	-0.025	0.877
Kidney Oxychlordane (ng/g)	-0.137	0.394
Liver Trans-nonachlor (ng/g)	-0.235	0.139
Liver Oxychlordane (ng/g)	-0.156	0.329
Brain Trans-nonachlor (ng/g)	-0.175	0.275
Brain Oxychlordane (ng/g)	-0.039	0.810
Tissue Trans-nonachlor (ng/g)	0.197	0.216
Tissue Oxychlordane (ng/g)	0.206	0.197

Discussion

The aim of this study was to determine concentrations of pesticides in human adipose tissue and serum samples from individual's adipose tissue in Iraq and evaluate some rationale for their occurrence and potential health risks based on the results; and up to our knowledge, this is the first comprehensive study of human adipose tissue and organs in the Iraq. The study was to verify the hypothesis that levels of the various lipid components (total cholesterol and tri-glycerides) are differentially associated with concentrations of trans-nonachlor and oxychlordane and do not have identical associations in serum samples obtained from post-mortem and antemortem cases in this cross-sectional study. Since levels of chlorinated pesticides change in direct

proportion to blood lipid levels, improper test interpretations can result from examining only the concentrations in blood. Measurement of cholesterol and tri-glycerides in the serum from the same specimen used to perform the testing allows calculation of total lipid level. The chlorinated pesticides concentrations can then be expressed as nano-gram per gram (ng/g) lipid [(15)]. Serum and different tissue organ samples were obtained from 41 post-mortem cases and serum and fatty tissue samples were also obtained from 40 antemortem cases who were not on any lipid-lowering medication and were analyzed for trans-nonachlor, oxychlordane, total cholesterol and triglyceride concentrations. Associations between toxicant concentrations and lipid levels were determined using multiple linear regression analysis. The study observed that elevated serum concentrations of lipids were positively associated with elevated serum concentrations of trans-nonachlor and oxychlordane pesticides in analyses adjusted for age and gender. The mean serum concentrations of triglycerides (153.75) was within "normal" ranges (triglycerides <200 mg/ml) while mean serum of the total cholesterol (209.89), which was elevated above normal range (120-200 mg/ml). Elevations in levels of trans-nonachlor, oxychlordane were associated with elevated levels of serum lipids. Since elevated serum lipids are a major risk factor for cardiovascular diseases, the previous association, if causal, may have significant effects on human health [(16)]. The strongest associations in antemortem cases were seen for trans-nonachlor compared with oxychlordane. There were significant positive and negative correlation between serum concentrations of trans-nonchlor and oxychlordane and their serum triglyceride and cholesterol. Positive and statistically significant correlations were observed between adipose tissue and serum concentrations of trans-nonachlor and oxychlordane but not of the remaining persistent organic pollutants, confirming reports that serum or plasma concentrations may not provide an accurate representation of concentrations in adipose tissue in all situations [(17)]. Reports on correlations between serum and adipose tissue concentrations range from negative values to coefficients above 0.8 [(18)]. Knowledge remains limited on relationships between serum and adipose tissue concentrations of persistent organic pollutants [(19)]. Concentrations in the two matrixes have different biological meanings: adipose tissue levels have been proven to be a good indicator of cumulated long-term exposure, whereas serum levels are considered a measure of current exposure and the mobilization of persistent organic pollutants from fatty tissues [(20)]. The study observed that elevated concentrations of trans-nonachlor more than oxychlordane in post-mortem tissue organs as well. The results reported in this study were consistent with conclusions made in study of a Native American population in which pesticide levels were found to be positively correlated with serum total cholesterol and triglyceride concentrations. The study showed that elevated concentrations of Trans-nonachlor and oxychlordane in postmortem tissue organs and antemortem tissue samples more than in serum. Since these chemicals are fat-soluble and tend to bioaccumulation in humans' tissues, they can cause a variety of health problems that often begin slowly. All fat-soluble toxins are carried in the lipid fraction of the serum, mostly in low-density lipoprotein particles (LDL). Since levels of chlorinated pesticides change in direct proportion to blood lipid levels, improper test interpretations can result from examining only the concentrations in blood. We have found significant positive associations between serum concentrations of (trans-nonachlor and oxychlordane) and their prevalence in a sample of Iraqi population after adjustment for age, sex and total concentrations of serum lipids. While these results do not prove cause and effect, they are consistent with the findings and conclusions of other studies. Serum concentrations of trans-nonachlor and oxychlordane were found to have strong associations with lipid components. The result showed elevated serum concentrations of lipids were significantly

associated with serum concentrations of organochlorines pesticides (trans-nonachlor and oxychlordane). Increased concentrations of organochlorine pesticides were associated with elevations in total serum lipids, total cholesterol and triglycerides. These observations showed selective effects of different organochlorines on serum concentrations of different groups of lipids. This elevation in concentrations of serum lipids may be attributed for the increased incidence of cardiovascular diseases found in persons with elevated exposures to chlorinated pesticides [21]. The study al-so showed value of total lipid in serum and elevated percentage of concentration of lipid to serum trans-nonachlor more than that of oxychlordane. The study revealed that levels of (trans-nonachlor and oxychlordane) in adipose tis-sue were more than levels in serum, because of half-life of organochlorines pesticides (trans-nonachlor and oxychlordane) in serum is few hours less than that for organochlorines pesticides (trans-nonachlor and oxychlordane), which are accumulated mainly in adipose tissue and their half-life continue for many years. There were elevated concentrations of trans-nonachlor in liver, brain and tissue more than in kidney and there were significant differences between them. There was elevated concentration of trans-nonachlor in brain more than other organs. There were also significant differences in the concentration of oxychlordane in liver, brain, kidney and fatty tissue. The study observed elevation of oxychlordane in adipose tissue more than in liver, brain and kidney and elevated concentration of oxychlordane in brain more than liver. The amounts stored in adipose tissue were the result of bioaccumulation of these toxins over a lifetime. However, since these chemicals are fat-soluble and tend to bioaccumulation in animals and humans, they can cause a variety of health problems that often begin slowly. The effects of these compounds are most often seen secondary to mitochondrial toxicity in neurological, immunological, and endocrinological systems; moreover, they can also affect the cardio-vascular, respiratory, gastrointestinal, and other systems in the body. Relation of these two organochloredane with age in each organ give negative correlation only in adipose tissue give positive correlation these mean the accumulation of organochlorines increase with increased of age [22].

This study concluded:

1. Traces of organochlorines (trans-nonchlore and oxychlordane) were detected in human serum and tissue indicating the presence of human contamination.
2. There was appositive correlation between both trans-nonchlor and oxychlordane with some lipid profile (Cholesterol and triglyceride.
3. Both trans-nonchlor and oxychlordane were higher in lipid tissue than serum and other tissue among postmortem cases.
4. Trans-nonchlore concentrations were higher than oxychlordane in all samples of the study.

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Authors Contribution:

Hmood: Collection of samples. Dr. Ali: Analysis and interpretation. Dr. Al-Qazzaz: Discussion.

Conflict of interest

The authors declare no conflict of interest.

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Correspondence to Harb S. Hmood
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