مجلة كلية التربية للعلوم الصرفة - جامعة ذي قار
المجلد ٨، العدد ٢، حزيران ٢٠١٨
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Evaluation MDA and Lipid Profilein Iraqi Women with UnexplainedInfertility

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Abstract:

The aim of this study is evaluation ofserum lipid peroxidation and levels of lipids profile in women with unexplained infertility (UI). 60 women with unexplained infertility as well as (40) women with polycystic ovarian syndrome (PCOS) aged (20-35) year were included in this study. The women were divided according the type of infertility into primary and secondary groups (2ºUI, 1ºUI, 1ºPCO and 2ºPCO) respectively. 50 healthy fertile women with the same age were included in this study as a control group. The results showed a significant increase in serum (MDA, TC, LDL, TG and VLDL) and decrease in serum (HDL) levels in all infertility groups compared to control group. Also, it is a significant increase in serum (MDA, TC, LDL, TG and VLDL) and decrease in serum (HDL) in (1ºPCO) and (2ºPCO) groups in comparison with (1ºUI), (2ºUI) respectively. This study shows that women with unexplained infertility had elevated TC, LDL, TG and VLDL levels, suggesting a higher risk of lipid peroxidation and a higher risk of developing CVD in the future

Keywords:Unexplained infertility, polycystic ovary syndrome,Oxidative stress, lipid profile

Journal of College of Education for Pure Sciences
Volume 8, Number 2, June 2018
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

تقدير(MDA) و صور الدهون في النساء العراقيات المصابات بالعقم غير المفسر

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الخلاصة

تهدف هذه الدراسة الى تقديرالاكسدة الفوقية للدهونو صور الدهون لدى النساء المصابات بالعقم غير المفسر. تضمنت الدراسة ستين امرأة مصابة بالعقم الغير مفسر(UI) واربعين امرأة مصابة بعقم تكيس المبايض (PCOS). قسمت النساء العقيمات الى مجاميع عقم اولي ومجاميع عقم ثانوي (1ºUI, 2ºUI, 1ºPCO 2ºPCO), كما تضمنت الدراسة خمسين امرأة خصبة صحيحة بنفس عمر النساء العقيمات كمجموعة سيطرة . بينت نتائج الدراسة ان هنالك ارتفاع معنوي (p>٠,٠٥) في مستويات (MDA, TG, VLDLLDL,TC)وانخفاض معنوي (p>٠,٠٥) في مستوى (HDL) في مصل جميع مجاميع العقم مقارنة بمجموعة السيطرة. كما بينت النتائج ان هناك ارتفاع معنوي (p>٠,٠٥) في مستويات (MDA,TG, VLDL,LDL,TC)وانخفاض معنوي (p>٠,٠٥) في مستوى (HDL) في مصل مجموعتي نساء تكيس المبايض (2ºPCO), (1ºPCO) مقرنة بمجموعتي نساء العقم الغير مفسر(2ºUI), (1ºUI). اظهرت الدراسة ارتفاع في مستويات(TC و LDLوTG وVLDL) في النساء اللواتي يعانين من العقم غير المفسر ، مما يوحي بوجود خطر أعلى للأكسدة الفوقية للدهون وهذا يؤدي الى زيادة خطر تطور أمراض القلب والأوعية الدموية لدى النساء اللواتي يعانين من العقم غير المفسر في المستقبل.

INTRODUCTION

Infertility is defined as the inability to conceive naturally after one year of regular unprotected intercourse. Most of the time, infertility is some degree of subfertility in which 1 in 7 couples need specialist help to conceive. Subfertility can be either primary or secondary.[1]Infertility of unknown origin comprises both idiopathic and unexplained infertility.[2,3] Most of the infertile couples have one of these three major causes including a male factor, ovulatory dysfunction, or tubal-peritoneal disease.[4] According to the Center of Disease Control, the causes of female infertility can be divided into three broad categories including defective ovulation, transport and implantation.[5]

Unexplained infertility is one of the controversial subjects in infertility on which agreement is rarely found among practitioners. It is a term used to define 30–40 % of couples in whom standard investigations including semen analysis, tests of ovulation and tubal patency have failed to detect any gross abnormality.[3] Couples with unexplained infertility suffer from both diminished and delayed fecundity.[6]The diagnosis of unexplained infertility may be frustrating because if there is no explanation for infertility, there is no effective treatment [6]. The prognosis is worse if the duration of infertility exceeds 3 years and female partner is > 35 years of age.[7] Treatment has been indicated if the duration is more than 2 years or the female partner is > 35 years of age.[7, 8]

Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, chronic anovulation and polycystic ovaries.[9, 10]The prevalence of classical forms of PCOS is from 6-9 to 19.9% and is even higher in women with menstrual disorders (17.4-46.4%), hyperandrogenism (72.1-82.0%), and anovulatory infertility (55-91%).[11,12] Clinical symptoms include ovulatory dysfunction, hyperandrogenism, and polycystic ovaries. Metabolic disorders (obesity, insulin resistance, impaired glucose tolerance, type II diabetes mellitus, and dyslipidemia) are typical of 40-85% women with PCOS.[13]

Generation of reactive oxygen species (ROS) is a normal feature of basal aerobic metabolism that supports life. They are active derivatives produced during the intermediate steps of oxygen reduction, which are catalyzed by small molecules such as iron and copper.[14]

Biological systems contain an abundant amount of O_2. Free radicals are often generated from O_2 and partially from normal metabolic processes in the body. They are unstable and highly reactive due to unpaired electrons that are capable of initiating an uncontrolled cascade of chain reactions, resulting in cellular damage and disease.[14]ROS are involved in physiological functions in female reproduction such as are oocyte maturation, ovarian steroidogenesis, ovulation, implantation, and formation of fluid filled cavity, blastocyst, luteolysis and luteal maintenance in pregnancy. ROS acts as mediators of various signaling path ways. Elevated or sustained generation of free radicals lead to

imbalance in the intracellular redox homeostasis. Excess levels of free radicals and ROS can be neutralized by antioxidants. Any imbalance between ROS and antioxidants can cause oxidative stress.[15] Oxidative stress involved in various pathologies of female reproductive tract like (PCOS) polycystic ovarian syndrome, endometriosis, tubal factor infertility, unexplained infertility, fibroids, recurrent pregnancy loss, spontanious abortions.[16] Lipid peroxidation is a self-propagating reaction unless it is counteracted by antioxidants. Malondialdehyde (MDA) is anmembrane. As hypoxia intensifies the peroxidation and cell membrane disruption, increase in extra cellular activity of Lactate dehydrogenase (LDH). LDH can be used as a hypoxia marker.[17]Follicular fluid contains high concentrations of anti-oxidants, which protects oocytes from ROS-induced damage. An imbalance in the pro-and anti-oxidant systems in the follicular fluid could lead to abnormal development of the oocytes and impaired fertility.[18]

Under normal conditions, scavenging molecules known as antioxidants convert H_2O_2 to H_2O to prevent overproduction of ROS. There are two types of antioxidants in the human body: enzymatic antioxidants and non-enzymatic antioxidants.[19, 20]Enzymatic antioxidants are also known as natural antioxidants;they neutralize excessive ROS and prevent it fromdamaging the cellular structure. Enzymatic antioxidantsare composed of superoxide dismutase, catalase, glutathioneperoxidase and glutathione reductase, which alsocauses reduction of hydrogen peroxide to water andalcohol[21]. Non-enzymatic antioxidants are also known as synthetic antioxidants or dietary supplements.[19]The body's complex antioxidant system is influenced by dietary intake of antioxidant vitamins and minerals such as vitamin C, vitamin E, selenium, zinc, taurine, hypotaurine, glutathione, beta carotene, and carotene.[22]

The influence of abnormal lipoprotein metabolism on female infertility has not been thoroughly explored, despite observations suggesting a potential role for plasma lipoproteins, especially HDL.[23, 24] Lipoproteins transport between tissues a number of lipids (e.g., cholesterol, steroid hormones, and vitamin E) that either directly, or indirectly through their metabolic products.[25]

In many species, including humans, the only lipoprotein detected in substantial amounts in the follicular fluid surrounding the developing oocyte in the ovary is HDL.[26, 27] HDL may deliver critical lipid nutrients to either the follicular cumulus cells or the oocytes for membrane synthesis, local steroid hormone production, or other processes essential for normal oocyte maturation. It might also have a role in cholesterol efflux[28,29,30]from the oocyte/cumulus cells, thus participating in the maintenance of cellular cholesterol balance.Therefore, abnormalities in HDL metabolism affecting its structure, abundance, or function might compromise female fertility.[31]

Dyslipidemia is the most common metabolic abnormality in PCOS, although the type and extent of the derangement has been variable. According to the National Cholesterol Education Program guidelines, about 70% of PCOS women have borderline or high lipid levels[32]. Ethnicity could represent an important determinant of the metabolic derangements in population. A recent Danish study on a significant number of women with PCOS showed differences for the median values of total cholesterol, LDL cholesterol and triglycerides, although obtained lipid values were within the range of normality.[33]

PATIENTS and METHOD

This study has been conducted at The infertility Unit inAL-Hussein Teaching Hospital in Thi-Qar, Biochemistry Laboratory in College of Science, at the period between 10/8/2016 to 25/5/2017. Informed consent was obtained verbally from all participants. A total of one hundred women with infertility of the ages 20 – 35 years. The women are already diagnosed as infertile women by the consultant medical staff, according to clinical examination and symptoms. Sixty women suffer from unexplained infertility whereas the forty women suffered from polycystic ovarian syndrome to get the normal values of studied parameters, the study included fifty healthy age matched fertile women with a history of at least one child birth were also enrolled.The details of the numbers and age of the two groups are illustrated in Table I

TABLE I**.** Details of numbers and age of the studied groups

Groups	No	Rang of Age (year)
Patients (infertile women)	100	20- 35
Control (fertile women)	50	20-35
Total	150	20-35

Also the women in this study were divided into subgroups according to type of infertility of infertile women showed as followings:

(1ºUI): included (30) woman with primary unexplained infertile women.

(2º UI): included (30) woman with secondary unexplained infertile women.

(1ºPCO): included (20) woman with primary explained infertile women (PCOS).

(2ºPCO): included (20) woman with secondary explained infertile women (PCOS).

Collection of Blood Samples

About (5mL) of blood samples was collected by vein puncture using asterile disposable syringe in plain plastic tubes. The serum was separated immediately in order to allow clotting at room

temperature. The blood was centrifuged at 3000 revolution per minute (rpm) for 10 minutes and stored in plain tubes at (-20ºC) until used or immediately analysed.

Determination of Serum Malondialdehyde (MDA)

The level of serum malondialdehyde was determined spectrophotometrically according to the method of Muslih *et al.*, 2002.[34] In brief; to 150 µl serum sample the following was added: 1ml of 17.5% trichloroacetic acid TCA provided by (BDH, England), and 1ml of 0.66% TBAprovided by (BDH, England), mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70% TCA was added and the mixture allowed to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, the supernatant was taken out for scanning spectrophotometrically at (532nm). The concentration of MDA calculated as follow:

$$MDA\left(\frac{\mu mol}{L}\right) = \frac{absorbance\ at\ 532}{L \times \varepsilon} \times D \times 10^6$$

L: light path (1cm)

ε: Extinction coefficient $1.56\times10^5\ M^1\ .cm^{-1} = 6.7$

D: Dilution factor = 1 ml Vol. Used in ref./0.15

Determination of Serum Lipid profile

Serum total cholesterol was measured according to Allan and Dawson, 1979.[35] The used reagents were supplied by Biolabo (France). Serum TG was measured according to Tietz*et al*., 1999.[36] The used reagents were supplied by Biolabo (France). Serum HDL was measured according to Lopes-Virella, 1977.[37] The used reagents were supplied by Biolabo (France). LDL and VLDL concentrations were measured according to Friedwald *et al.*, 1972,[38] as follows:

LDL (mg/dl) = Total cholesterol – (HDL + VLDL)

VLDL (mg/dl) = serum TG /5

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Results And Discussion

The women with primary and secondary unexplained infertility (UI) had the mean age of 29.25 ± 6.26 and 33.69 ±6.36 years respectively, and the mean age of women with primary and secondary

explained infertility (PCOS) groups were 25.844 ± 4.47 and 28.44± 4.21 years respectively. The mean age of the control group was 32.28±6.70 years. The women with primary and secondary (UI) had the mean Body mass index BMI values of 26.44±3.24 and 27.27±4.04 kg/m^2 respectively, and the mean BMI values of women with primary and secondary (PCOS) were 30.77±3.11 and 31.34±4.12 kg/m^{2,} respectively. The mean BMI value of control group was 64.02 ± 6.96 kg/m^2.

Serum Malondialdehyde Concentration

The results of Table (II) showed a significant increase in concentration of serum MDA in all women with unexplained infertility (UI) and explained infertility (PCO) in comparison with control groups ($p \leq 0.05$). The same Table shows a significant increase in concentration of serum MDA in the two groups of woman with explained infertility (1ºPCO) and (2ºPCO) in comparison with the two groups of women with unexplained infertility (1ºUI) and (2ºUI) respectively ($p \leq 0.05$). However, there are no significant difference in concentration of serum MDA between (1ºUI) and (2ºUI) groups as well as (1ºPCO) and (2ºPCO) groups ($p \leq 0.05$),can be observed.

These results are agreed with the studies ofSavita *et al.* 2009; Al.Mukhtar *et al.* 2012 andALL-Ahmed *et al.* 2015.[39, 40, 41] In the human body, reactive oxygen species (ROS) are formed under physiologic and pathologic condition.[42, 43] They can be produced from endogenous sources, for instance during aerobic metabolism and due to different metabolic pathways, or as part of defense mechanism of the body. In addition, ROS can be formed exogenously as a result of numerous environmental pollutants and by cigarette and alcohol use.[42] Data have been implicated that regulated levels of ROS in ovaries, endometrium, fallopian tube, embryos, and peritoneal fluid play a role in tissue remodeling, hormone signaling, ovarian steroidogenesis, folliculogenesis, maturation of oocyte, tubal function, and cyclical and endometrial changes.[21] The high rate of free radicals production in female with unexplained infertility may be generated from increases metabolism and depletion of protective antioxidants.[40]

The results showed increase in concentration of serum MDA in the two groups of women with explained infertility in comparison with fertile women. These results are agreement with the resultsof Deepika *et al.* 2014; Sumithra *et al.* 2015 andZahoorunnisa *et al.* 2017.[44, 45, 46] But there are no a significant increase in the two groups of women with explained infertility (1ºPCO) and (2ºPCO) in comparison with the two groups of woman with unexplained infertility respectively ($p \leq 0.05$). Theseresults in agreement with the results reported byDiamond*et al.* 2017.[47]Oxidative stress is also intimately involved in PCOS pathogenesis, since PCOS patients show more serious Oxidative stress compared with the normal.[48] In addition, oxidative stress is involved in the pathological processes of

Insulin resistance IR, hyperandrogenemia, and obesity as well, which accompany PCOS frequently but not absolutely.[49]

TABLEII.Serum MDA levels of control and patients groups

Groups	NO	MDA(*μmol*/) *mean± SD*
Control	50	2.06 ± 0.33 [c]
1º UI	30	3.06 ± 0.36 [b]
2º UI	30	3.11± 0.32 [b]
1º PCO	20	3.56 ± 0.97 [a]
2º PCO	20	3.88 ± 0.99 [a]
LSD		0.34

-Each value represents mean ± S.D values with non-identical superscript (a, b or c …etc.) were considered significantly differences (P ≤ 0.05).Control: fertile woman;1ºUI: Primary unexplained infertility;2ºUI:Secondary unexplained infertility;1ºPCO: Primary explained infertility;2ºPCO: Secondary explained infertility, No: Number of subjects;SD: Standard deviation;LSD:Least Significant Difference.

Serum Lipid Profile Concentrations

Table (III) shows a significant increase in the concentration of serumcholesterol and (LDL) and decrease inthe concentration of serum (HDL) in the two groups of women with unexplained infertility (1ºUI) and (2ºUI) in comparison with control group (p≤0.05). Also it is a significant increase in the concentration of serumcholesterol in (2ºUI) group in comparison with (1ºUI) group (P≤0.05). Whereas there are no significant differences in the concentration of serum (LDL) and (HDL) between the two groups of women with (1ºUI) and (2ºUI) (P≤0.05).

The same Table shows a significant increase in the concentration of serumTC and (LDL)and decrease inthe concentration of serum (HDL) in the two groups (1ºPCO) and (2ºPCO) in comparison with the control group (p≤0.05). Also it is a significant increase in the concentration of serumcholesterol and (LDL) in the two groups of women with explained infertility (1ºPCO) and (2ºPCO) in comparison with two groups of women with unexplained infertility (1ºUI) and (2ºUI) respectively (P≤0.05). But there are no significant differences in the concentration of serum (TC), (LDL) and (HDL) between (1ºPCO) and (2º PCO) groups (P≤0.05), can be observed.

The resultsshow a significant increase in the concentration of serum TG and VLDL in the two groups of unexplained infertile women (1ºUI) and (2ºUI) in comparison with the control group (p≤0.05). Whereas no significant differences in the concentration of serum TG and VLDL in the two groups of unexplained infertile women (1ºUI) and (2ºUI) (p≤0.05).Also, it is a significant increase in the concentration of serum TG and VLDL in the two groups of explained infertile women (1ºPCO) and (2ºPCO) in comparison with the control group (p≤0.05). The results show a significant increase in the concentration of serum TG and VLDL in the two groups of explained infertile women (1ºPCO) and (2ºPCO) in comparison with the two groups of unexplained infertile women(1ºUI) and (2ºUI) respectively. However there are no significant differences in the concentration of serum TG and VLDL between the two groups of explained infertile women (1ºPCO) and (2ºPCO) (p≤0.05), can be observed.

In this study, it is TG, TC, LDL, and VLDL levels are elevated and HDL is decreased in the women with unexplained infertility in comparison with control group (p≤0.05). These results are in agreement with the study of Verit *et al.* 2017[50]. It is widely known that infertility has been suggested to be a risk factor for CVD [51]. 50% increased risk of myocardial infarction or coronary heart disease was found among women with menstrual irregularities in comparison with those who had normal cycles from the ages of 20 to 35 years. Obesity, insulin resistance, and hypothyroidism are also related with infertility [51,50], anovulation, and CVD.[52]

Advanced maternal age, minimal to mild endometriosis, endocrine abnormalities, diminished ovarian reserve, and oxidative stress have been identified as possible etiological factors for unexplained infertility. It is widely known that CVD increases with age. Endometriosis, another common cause of unexplained infertility, is characterized by local and systemic chronic inflammation, dyslipidemia, and oxidative stress.[53] The two studies have shown that aging, obesity, dyslipidemia, and chronic inflammation are related with reduced oocyte quality and quantity.[54, 55]

This study, it is TG, TC, LDL, and VLDL levels are elevated and HDL is decreased in the women with explained infertility (PCOS) in comparison with control group (p≤0.05) these result are in agreement with the studies of Latha *et al.* 2012;George and Malini, 2014;Shah *et al.* 2017.[56, 57, 58]Also it is show TG, TC, LDL, and VLDL levels are elevated and HDL is decreased in the women with explained infertility (PCOS) in comparison with the women with unexplained infertility (UI) respectively (P≤0.05).These results are agreement with the results ofMichael *et al.* 2017.[59]

Elevated body mass index, cholesterol, TG, LDL levels and low level of HDL are associated with the risk for the development of metabolic syndrome [60]. This study showed deranged lipid profile in PCOS women with the findings of elevated TC, TG, VLDL and LDL levels and low HDL levels

supporting the existing knowledge ofVinita and Monini[61] Kumar *et al.*[62]and Naidu *et al.* [63] which indicated that dyslipidemia is prevalent in women with PCOS. The present study showed significantly raised cholesterol and LDL in PCOS women and is similar to the findings of ThathapudiSujatha*et al.*[64]but contradictory to the findings of Rasool Suzan Omer.[65]Metabolic transformation of LDL cholesterol by increased oxidation leads to higher atherogenic potential of modified LDL particles.[66, 67]To date, it is established that thyroid hormones promote the activity of 3-hydroxy-3methylglutaryl-coenzyme A (HMG-CoA) reductase. They also upregulate the LDL receptor gene.[68] The reduction of HMG-CoA reductase activity that accompanies hypothyroidism results in altered total cholesterol and LDL levels.[69]

Women with PCOS would be predicted to be at high risk for dyslipidemia because they have elevated androgen levels and are frequently obese. PCOS women are often hyperinsulinemic and insulin resistant, which can lead to dyslipidemia. Elevated plasma insulin concentrations enhance very-low-density lipoprotein (VLDL) synthesis, leading to hypertriglyceridemia. Progressive elimination of lipid and apolipoproteins from the VLDL particle leads to an increased formation of intermediate-density and low-density lipoproteins, both of which are atherogenic.[70, 56]

If the concentration of VLDL-transported triglyceride is high, cholesteryl ester transfer protein CETP promotes the transfer of LDL cholesteryl ester or HDL cholesteryl ester in exchange for triglyceride.Triglyceride-rich HDL cholesterol or LDL cholesterol can undergo hydrolysis by hepatic lipase or lipoprotein lipase.[71]

TableIII.Serum lipid profile levels of control and fertile women groups.

Groups	No	TC **mg/dl** mean± SD	LDL **mg/dl** mean± SD	HDL **mg/dl** mean± SD	**TG mg/dl** mean± SD	VLDL**mg/dl** mean± SD
control	50	141.51±16.29 ^{d}	72.30 ± 23.71^{c}	50.43±13.59^{a}	91.98±21.36 ^{c}	18.78±4.97^{c}
1º UI	30	150.22 ± 19.36^{c}	93.11±20.35 ^{b}	35.69± 6.74^{c}	105.33±28.74^{b}	21.43±5.65^{b}
2º UI	30	158.61 ± 17.31^{b}	92.80±22.59 ^{b}	41.87± 6.13^{b}	116.85±23.47^{b}	23.94±5.05^{b}
1º PCO	20	175.23± 16.88^{a}	103.65±18.87^{a}	44.91± 4.83^{b}	133.10±34.42^{a}	26.67±6.87^{a}
2º PCO	20	174.26±14.50^{a}	104.28±14.25^{a}	42.97 ± 7.82^{b}	134.79±33.42^{a}	27.01±6.66^{a}
LSD		7.13	8.41	3.54	11.90	2.44

- Legend as in Table II

Conclusions

This study concluded that is the women with unexplained and explained infertilityhad elevated TC, LDL, TG and VLDL levels, suggesting a higher risk of lipid peroxidation and a higher risk of developing CVD in the future

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