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Serum vitamin D and TNF-αin Iraqi infertile women with positive IgG toxoplasma gondii: Is there a correlation between infertility and vitamin D deficiency.

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ABSTRACT

Vitamin D is strongly associated with fertility proplems in women. There is also evidence of its essential role for the proper functioning of the human. This study aims to evaluate the relationship between vitamin D and TNF-α in women's fertility. We sought to determine vitamin D in woman attending Kammal El-Sammarai hospital from november 2016 to april 2017 .Fifty women with reproductive failur participated in the study classified as 25 infertile women with positive IgG toxoplasma gondii and 25 infertile women with negative IgG toxoplasma gondii .Serum levels of Vitamin D and TNF-alpha were done by Enzyme linked Immuno Sorbent Assay (ELISA) ,in addition to lipid profile determination .ALL these parametrers were compared with age ,body mass index of thirty fertile women. There is a significant decrease($p<0.011$)in vitamin D levels in infertile women with positive IgG toxoplasma gondii while TNF-alpha showed significant increase ($p<0.001$)as compared with fertile group. Vitamin D levels were further categorized into deficiency (vit. D3 < 5 ng/ml)or insufficiency (vit. D3 = 20-29 ng/ml), sufficiency (vit. D3 ≥30 ng/ml). The inter group comparison showed highest values of TNF- alpha in vitamin D defficiency group(<20 ng/ml) of infertile women with positive IgG toxoplasma gondii with correlation coefficient($r = -0.715$, $p< 0.002$).A positive correlation found between HDL-Cholestrol with vitamin D while its correlated negativly with body mass index in all studied groups. Vitamin D deffeciency (<20 ng/ml) is most frequently in infertile women with positive IgG toxoplasma gondii and its acompined with high levels of TNF – alpha.

Keywords: Toxoplasma gondii, Vitamin D, infertility, cytokine, lipid profile.

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INTRODUCTION

Toxoplasmosis, caused by the protozoan parasite *T. gondii*, is one of the most common parasitic infections of human and other warm-blooded animals. Approximately 50% of the humans are infected and developed a disease named toxoplasmosis, which is one of the most common parasitic zoonoses throughout the world. In different regions within any country and among different population groups based on various social, cultural lifestyle, and environmental factors, the prevalence of *T. gondii* always varies [1].Toxoplasmosis manifests no clinical signs in 80% of cases in immune competent patient, causing immunization characterized by the persistence of cysts, particularly in brain, muscles, and retina. Assessing the serological status, based on testing for serum toxoplasma IgG and IgM antibodies, is essential in cases that are increasingly at risk for the more severe disease forms, such as congenital or ocular toxoplasmosis. This disease also exposes immune suppressed patients to reactivation, which can lead to more widespread forms and increased mortality [2].

Infertility is a health trouble that affects couples worldwide, regardless of their ethnicity, society, culture or economic status[3]. Infertility described as a condition manifested through the inability to conceive and to realize a successful clinical pregnancy after 12 months of regular and unprotected sexual intercourse[4]. A recent study found an association between toxoplasmosis and infertility. This finding encourages both prompting health education to prevent *Toxoplasma* infection in female population especially in childbearing age and further investigation to elucidate the causative relation between *T. gondii* infection and female infertility. [5].

Vitamin D is a fat‑soluble vitamin and a pro hormone which has two isoforms, ergocalciferol (Vitamin D2) available from plant sources and cholecalciferol (Vitamin D3) produced by animals. In humans, more than 80% of the total Vitamin D of the body store is synthesized cutaneously through sunlight (ultraviolet B radiation) exposure while rest is obtained from the diet[6,7].Vitamin D deficiency has been recognized as an international public health problem due to its important role in health and disease, mainly for the skeletal system where vitamin D deficiency causes rickets, osteomalacia, and osteoporosis [8]. Even in countries with plentiful sunshine, epidemic prevalence of vitamin D deficiency has been reported in the general population and especially in women and children [9]. Data accruing from studies undertaken either in animals or humans point to a potential role of vitamin D in female fertility [10].In women, vitamin D deficiency may also be involved in the pathogenesis of infertility, menstrual dysfunction, and menstrual abnormalities[11].

Cytokines are very important regulatory molecules not only for the immune system, but for many other significant functions of the organism. During ovulation, follicular rupture occurs due to the production of the plasminogen activator with both TNF-α and IL-1b by simutaneously activation of gonadotropns (follicular stimulating hormone-FSH- and luteinizing hormone-LH-). The plasminogen activator pathway goes through activation of plasmin. In turn, plasmin stimulates collagenases, which actually induce rupture of the follicle and ovulation [12].

The stimulatation of TNF-α and IL-1b led to the production ofIL-8 and Granulocyte-macrophage colony stimulating factor(GM-CSF). Cytokines like IL-8 and GMCSF, will further facilitate ovulation and embryo implantation [13].

Serum 25(OH) vitamin D status is inversely related to TNF-α concentrations in healthy women, which may in part explain this vitamin's role in the prevention and treatment of inflammatory diseases. [14].Vitamin D status and its relationship with lipid profile is unclear. In some studies, the positive effect on lipid profiles has been proposed for vitamin D, but it is not clear whether or not the beneficial effects of vitamin D are due to the hormone itself or its association with calcium metabolism [15, 16]. Calcium acts to form insoluble soaps with dietary fat, preventing its absorption, thus modulating the effect of high dietary fat on blood lipid concentrations [17].

but other studies have not demonstrated the role of vitamin D supplementation in improving lipid profile[18,19]. Thus, our overall objective was to study the relationship between vitamin D status (as determined by serum 25(OH)vitamin D concentrations) with inflammatory marker (TNF-α) in fertile and infertile Iraqi women with or witout positive IgG toxoplasma gondii and to examine their correlation with lipid profile.

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MATERIALS AND METHODS

Subjects and anthropometric measurements

The present study was carried out on a total number of 80 Iraqi women with age ranged from (20 – 41 years). The study participants were divided into two groups, the first group consist of 50 infertile women classified into two groups: twenty five infertile women with positive toxoplasma gondii (PP) and twenty-five infertile women with negative toxoplasma gondii (NP). The second group consist of 30 healthy fertile normally ovulating women having unless one baby matched for age and body mass index (BMI) served as control group. The second group were also classified into two groups: fifteen fertile women with positive toxoplasma gondii (PC) and fifteen fertile women with negative toxoplasma gondii (NC). The incidence of positive toxoplasma gondii considered as IgG > 40 IU/ml while the negative toxoplasma gondii considered as IgG < 30 IU/ml**.** All of the samples were found sero negative of IgM. All patients were attending to Kammal El-Sammarrai Hospital in Iraq during the period of November 2016 to Aprile 2017, they were fasting after midnight before blood collection in the next morning.

All females had been married for at least two years and were being treated for primary infertility. Females were chosen as they have normal sexual life and did not take any contraceptive measures. A special questionnaire that contains all required informations was completed for each patient, prior to blood sampling. Patients with other causes of infertility (ovarian, tubal, galactorrhea, hormonal, infection, taking any hormonal medication, and abortion) were excluded from the study. In addition to that, females who their couples are infertile were excluded from the study. Detection of anti- *Toxoplasma gondii* antibody (IgG- and IgM) by Enzyme linked Immuno sorbent Assay(ELISA) technique using bio Check *Toxoplasma*kit .

Blood Samples Collection

Fasting whole blood was collected (6 - 8 ml) from infertile and fertile women, kept in tube without any anticoagulant at room temperature for 1 hour. Then each tube was centrifuged (2000×g) for 10 minutes, the clear serum was pipetted into clear dry test tube and then stored at (-20) ^{o}C for subsequent analysis. The stored serum utilized for different metabolic parameter, body mass index, 25(OH) vitamin D, TNF-α and lipid profile.This study was approved by the human research ethics committee of the hospital, and informed consent was obtained from each patient.

Measurment Of Bmi

Weight and height were measured and BMI was calculated by dividing weigh in (Kg) by squire of height in (m).

Estimation Of 25(Oh) Vitamin D (Vit D)

Competitive immunoassay ELISA kit is used for quantities of vit D level, (Monobind Inc.) (Lake Forest, CA 92630, USA) according to the manufacture protocols. Endocrine Task Force guidelines define vitamin D deficiency as a 25(OH)VitD level of <20 ng/ml, vitamin D insufficiency as a 25(OH)VitD level of 21-29 ng/ml and vitamin D sufficiency as a 25(OH) VitD level of ≥30 ng/ml. [20].

Estimation of TNF-α

Sandwich enzyme immunoassay ELISA kit is used for quantities of TNF-α level,**(R&D SYSTEMS abiotechne brand, UK & Europe)**according to themanufacture protocols. The determination of TNF-α present in the sera sample based on the principal of sandwich enzyme immunoassay binding.

Estimation of lipid profile

According to the manufacture protocols, serum triglycerides assay was done by enzymatic colorimetric tests with glycerol phosphate oxidase, (LiNEAR Chemicals .s.L.). Total serum cholesterol was assayed by enzymatic colorimetric tests with cholesterol esterase and cholesterol oxidase, (LiNEAR Chemicals.s.L.). As well as HDL-cholesterol was measured after precipitation of the apolipo protein B-

containing lipoproteins with phosphotungstic acid, (Biosystems). Low-density lipoprotein cholesterol was calculated by the Friedewald formula [21].

Statistical analysis

Computer IBM SPSS software package version 22.0was use for data statistical analysis. In this study the data was presented as Mean± Standard deviation (Mean± SD) using Independent-samples T-Test to compare the mean. A value of ($p<0.05$) &($p<0.001$), were considered as statistically significant, & highly significant respectively.

RESULTS

In our study,80 Iraqi women were divided into four groups, positive IgG infertile patient group (PP, n=25),negative IgGinfertile patients group (NP, n=25), positive IgG control group(PC, n=15)and negative IgG control group(NC, n= 15).

The results of our study showen in **Table (1)**revealed that BMI showed slightly significant different (p= 0.049) in NP group as compared to NC group (27.72 ±4.1 Kg/m^{2}vs. 25.36±2.33Kg/m^2). Serum vitamin D level showed no significant different (p=0.094) in NP group as compared to NC group (21.97±8.84ng/mlvs. 26.68±7.58 ng/ml), while, serum TNF-α showed highly significant increase (p=0.000) in NP group as compared to NC group (21.08±3.15pg/mlvs. 16.88±3.69 pg/ml). The results of lipid profile showed that, there were insignificant differences in mean (cholesterol, TG, LDL-C and VLDL-C) levels of NP group in comparison with NC group (p=0.853, 0.145, 0.987 and 0.139 respectively). While serum level of HDL-C was showed a significance decrease(36.81±10.55mg/dLvs 48.93±9.80mg/dL,p=0.001) in NP group as compared to NC group.

In addition to that **Table (1)**showed no significant increase in the mean of BMI in PP group (p=0.494) as a compared to PC group (27.57±4.09Kg/m^2 vs. 26.59 ±4.74 Kg/m^2).A significant decrease of vitamin D is obvious in serum of (PP) group (p=0.011) as compared with (PC) group (18.84 ±8.46ng/ml vs. 24.84 ±2.6ng/ml), while a highly significant increase in TNF- **α** in PP group (p=0.001) in comparison to PC group (19.22±3.65pg/ml vs. 15.33±2.55 pg/ml) .Mean of serum HDL-C showed a highly significant decrease in PP group (p=0.001) as compared to PC group (34.78 mg/dL vs. 45.51 mg/dL), and there was a no significant increase in mean (cholesterol, TG, LDL-C and VLDL-C) levels of PP group in comparison to PC group (p=0.136, 0.827, 0.578 and 0.812respectively) . The results presented in **Table (2)**clear that there is no significant difference in the parameters (BMI, vitamin D, TNF-α, Cholesterol, TG, HDL-C, LDL-C and VLDL-C) in serum of PC group in comparison to the NC group.

Table 1: The characteristics of participants of BMI, vitamin D, TNF-α and lipid profile among different groups (n=80).

Variables	NC (n=15) (Mean±SD)	NP (n=25) (Mean±SD)	*P*	PC (n=15) (Mean±SD)	PP (n=25) (Mean±SD)	*P*
BMI (Kg/m^2)	(25.36±2.33)	(27.72±4.1)	0.049*	(26.59±4.74)	(27.57±4.09)	0.493
Vit.D (ng/ml)	(26.68±7.58)	(21.97±8.84)	0.094	(24.84±2.6)	(18.84±8.46)	0.011*
TNF-α (pg/ml)	(16.88±3.69)	(21.08±3.15)	0.000**	(15.33±2.55)	(19.22±3.65)	0.001**
Cholesterol(mg/ dL)	(171.52±41.13)	(169.24±34.82)	0.853	(179.35±40.04)	(160.92±35.76)	0.136
Triglyceride(mg/ dL)	(107.45±26.90)	(128.3±49.98)	0.145	(106.79±35.88)	(104.50±29.0)	0.827
HDL-C(mg/dL)	(48.93±9.80)	(36.81±10.55)	0.001**	(45.51±11.84)	(34.78±7.97)	0.001**
LDL-C(mg/dL)	(106.56±55.54)	(106.77±28.43)	0.987	(112.11±39.34)	(105.23±36.39)	0.578
VLDL-C(mg/dL)	(21.49±5.38)	(25.72±9.96)	0.139	(21.30±7.17)	(20.81±5.76)	0.812

***The difference is significant at the 0.05 level.**

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Results were expressed as mean ± SD.BMI=body mass index; Vit.D= 25(OH)vitamin D; TNF-α=Tumour necrosis factor-alpha;;HDL=high-density lipoprotein; LDL=low-density lipoprotein; VLDL=very low-density lipoprotein.

Table 2: Values of BMI, vitamin D, TNF-α and lipid profile in positive IgG toxoplasma gondii among negative IgG toxo plasma gondii(n=80).

Variables	NC (n=15) (Mean±SD)	PC (n=15) (Mean±SD)	*P*	NP(n=25) (Mean±SD)	PP (n=25) (Mean±SD)	*P*
BMI(Kg/m^2)	(25.36±2.33)	(26.59±4.74)	0.376	(27.72±4.10)	(27.57±4.10)	0.896
Vit. D(ng/ml)	(26.68±7.58)	(24.84±2.6)	0.382	(21.97±8.84)	(18.85±8.84)	0.209
TNF-α(pg/ml)	(16.88±3.68)	(15.33±2.55)	0.192	(21.08±3.15)	(19.22±3.65)	0.059
Cholesterol(mg/dL)	(171.52±41.13)	(179.53±40.04)	0.593	(169.24±34.82)	(160.92±35.76)	0.408
Triglyceride(mg/dL)	(107.45±26.90)	(106.77±35.88)	0.955	(128.30±49.98)	(104.50±29.0)	0.045*
HDL-C(mg/dL)	(48.93±9.81)	(45.51±11.84)	0.396	(36.81±10.55)	(34.79±7.97)	0.447
LDL-C(mg/dL)	(106.55±55.54)	(112.11±39.34)	0.754	(106.77±28.43)	(105.23±36.39)	0.868
VLDL-C(mg/dL)	(21.49±7.17)	(21.30±7.17)	0.936	(25.72±9.96)	(20.80±5.76)	0.038*

*The difference is significant at the 0.05 level.

As well as results presented in **Table (2)** showed that, there were no significant differences in mean (BMI, vitamin D, TNF-α, cholesterol, HDL-C and LDL-C) levels of NP group in comparison with PP group, while serum level of TG and VLDL-C were show a significance increase (*p*=0.045and 0.038) respectively in NP group as compared to PP group.

Vitamin D showed a significant negative correlation with BMI while it correlated positively with HDL-C in all study groups, **Table (3)**. In addition,BMI showed a negative correlation with HDL-C in all studied groups while it correlated differently with other parameters of lipid profile, Table**(4)**.

Deficiency of vitamin D when its level <20 ng/ml, insufficiency as level of (21-29) ng/ml and sufficiency as level of ≥30 ng/ml. [20].Vitamin D levels in our studied groups were further categorized according to the above levels. In our study we observed that among the studied groups(n=80), 30 (37.5%) women have vitamin D deficiency, 36 (45%) women have vitamin D insufficiency and 14 (17.5%) women have sufficient amount of vitamin D, **Table (5)**.

Table 3: Pearson correlation of vitamin D in NP, PP, PC, and PP groups

Parameters	Vitamin D (ng/ml)							
	NC		PC		NP		PP	
	r	*P*	r	*P*	r	*P*	r	*P*
BMI (Kg/m^2)	-0.89**	0.000	-0.541*	0.037	-0.423*	0.035	-0.758**	0.000
HDL-C (mg/dl)	0.621*	0.006	0.532*	0.041	0.584**	0.002	0.490*	0.013

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

Table 4: Pearson correlation of BMI in NP, PP,PC, and PP groups.

Parameters	BMI (Kg/m^2)							
	NC		PC		NP		PP	
	R	*P*	r	*P*	r	*P*	r	*P*
Cholesterol (mg/dL)	0.098	0.729	0.580*	0.023	0.394	0.052	0.177	0.396

Triglyceride(mg/dL)	-0.003	0.993	0.741**	0.002	0.424*	0.034	0.012	0.954
HDL-C (mg/dL)	-0.672**	0.006	-0.648**	0.009	-0.401*	0.047	-0.413*	0.040
LDL-C (mg/dL)	0.075	0.791	0.681**	0.005	0.483*	0.014	0.264	0.201
VLDL-C (mg/dL)	-0.003	0.993	0.743**	0.001	0.431*	0.031	0.014	0.948

* Correlation is significant at the 0.05 level
**. Correlation is significant at the 0.01 level

Table 5: The distribution of women according to the levels of vitamin D.

Groups	Vitamin D (ng/ml)		
	<20(deficiency)	21-29 (insufficiency)	≥30(sufficiency)
studied groups (n=80)	30 (37.5%)	36 (45%)	14 (17.5%)
NC(n=15)	4(26.6%)	5(33.3%)	6(40%)
PC(n=15)	1(6.6%)	14(93.3)	---
NP(n=25)	9(36%)	12(48%)	4(16%)
PP(n=25)	16(64%)	5(20%)	4(16%)

From **Table (5)** we have concluded that most infertile women have defecient levels of vitaminD while less of them havesufficient levels of vitamin D. Vitamin D deficiency, determined as serum 25-hydroxyvitamin D levels <20 ng/ml, is estimated to affect about 50% of the population worldwide [8]. Vitamin D defeciency in PP group (n=16) correlated negativly with TNF-α (r =-0.715**), while no correlation observed in NP group (n=9), **Table (6)**.

Table 6: Corelation parameters of Vitamin D defeciency with TNF- α in NP and PP groups

Prameter	Vitamin D (ng/ml)			
	NP(N=9)		PP(N=16)	
TNF-α(pg/ml)	r	*P*	r	*P*
	0.092	0.813	-0.715**	0.002

**. Correlation is significant at the 0.01 level

DISSCUSION

In Iraq, Toxoplasmosis prevalence was determined in different governments and the seropositivity had been shown to be at different percentage of infection [22- 24].
Previous research on laboratory animals reported that infection with *T.* gondii could be a cause of infertility in experimental animals [25].Zhou et al. found that *Toxoplasma* infection in infertile human couples was higher than that in fertile ones[26].

Women *with T. gondii* positive reported to take a significantly longer time to conceive and to have more frequent or more serious fertility problems than *T. gondii*-free women. Infected *T. gondii*-women became pregnant at an older age, more often needed *in vitro* fertilisation and reported to take a longer time to conceive and to have more fertility problems than *T. gondii*-negative women[27].

These results support the hypothesis that latent toxoplasmosis has some negative effects on the reproductive capacity of *T. gondii* infected women. Akarsu et al. have suggested that *T. gondii*-associated infertility mechanisms include development of endo metritis and foetal rejection due to local release of *T. gondii* from cysts located in the endometrial tissue on stimulation during placenta formation, impaired folliculo

genesis in the ovaries and uterine atrophy and reproductive failure due to hypothalamic dysfunction as a result of chronic toxoplasmosis[28].

There are a large number of *in vitro,* animal as well as human observational studies which strongly point towards an association between vitamin D and female fertility. Research data indicate that vitamin D might be implicated in the pathogenesis and prevention of endometriosis, while vitamin D status has been linked to IVF outcome [29].

Vitamin D status were measured in serum of (NC& NP) groups, and the results in **(Table 1)** revealed that in spite of the presence of differences but it was not significant (p=0.094). While vitamin D status in serum of (PP) group was obvious a significant decrease (p=0.011) compared with (PC) group. Alessio et al. [30] found that women who had sufficient levels of vitamin D were more likely to produce high-quality embryos and more likely to become pregnant than women who were deficient in vitamin D.

Although several causes are responsible for infertility in both genders, vitamin D show a relationship with reproductive physiology in a study performed by Luk et al. [31]. Human and animal data propose that low vitamin D status is associated with impaired infertility, endometriosis, and polycystic ovary syndrome [32]. Defficiency of vitamin D may be contributed to the reduced exposure to sunlight with a shift in lifestyle, and increased use of sunscreen to prevent the effects of carcinogens from sun radiation [33].

Cytokines play a critical role in defense against the infection and are important in the pathogenesis of toxoplasmosis and toxoplasmic encephalitis. In vitro researches show that the vitamin D serum levels have impact on the cytokine profile in the organism [34,35].In our study a highly significance increase in TNF-α level (p=0.000) was detected in serum of (NP) group **(Table 1)** compared with control group (NC). Serum TNF-α level also showed a significance increase (p= 0.001) in (PP) group compared with (PC) group.

Our results are in line with that of Arck et al [36]and Clark et al [37] showed that TNF-α has both an anti-reproductive effect and an anti-embryonic effect that trigger off foetal loss. This observation contradicts wih other reserchers which reaveled that TNF-α had no significant differences in the concentrations between the infertile and the control (fertile) groups [38, 39].The normalcy in their result may be due to the different status of infertility of the women studied or the different causes of the infertility in these women. Other study indicates elevated level ofTNF-alpha and IFN-gamma found in women who suffer recurrent spontaneous abortions (RSA) or have infertility of unknown aetiology[40].

Body mass index was measured in the present study, and the results in **(Table 1)** show a significant increase (p<0.05) in infertile women with negative toxoplasma gondii (NP) group compared to that of fertile women with negative toxoplasma gondii (NC) group. These result of (NP) group is in line with Pasquali et *al*.[41], who found that female obesity is associated with higher risk of pathological endocrine conditions, such as infertility. Also, this compatible with the findings of Clark et al. [42] who found that obesity influences negatively the outcome of medical treatments for infertility.

There is an inverse association of serum 25(OH) vitamin D and body mass index (BMI) greater than 30kg/m^2, and thus, obesity is associated with vitamin D deficiency. [20].These finding may be due to that obese women unwillingness to expose their bodies to the sun thus led to decreased levels of vitamin D[43].

Our results indicate no significant differences in TNF- α levels in all studied groups upon comparison with their BMI. La vignera *et al*. evaluated the concentrations of TNF-α in the follicular fluid of obese women undergoing a medically assisted procreation cycle they found that patietns with a BMI between 35 and 39.9 kg/m^2 showed intrafollicular TNF-α levels significantly higher (p <0.05) compared to remaining groups [44].

An association between vitamin D and serum lipid concentrations in our studied grous was observed. A significant decrease of serum HDL-C in NP and PP groups (p=0.001), in comparison to NC and PC groups respectively and there was no significant difference in serum levels of cholesterol, TG, LDL-C and VLDL-C. It has been unequivocally proven that fat is metabolically active; as a result of lipolysis, the release and production of a number of pro inflammatory cytokines occur [45, 46].These finding disagreement with TNF-α levels in serum of all our studied group whish showed no correlation with lipid profile or BMI.In addition (BMI, vitamin D3,

TNF-α, cholesterol, TG, HDL-C, LDL-C and VLDL-C) showed non-significant difference (p>0.05) in serum of (NC) group **(Table 2)** compared to that of (PC) group. Also **(Table 2)** revealed that no significant difference was found (p>0.05) in serum levels of (BMI, vitamin D, TNF-α, cholesterol, HDL-C and LDL-C) in (PP) group compared to that of (NP) group, while serum level of (TG, VLDL-C) was show a significance decrease (p<0.05) in (PP) group compared to that of (NP) group. These differences may be due to that (64%) of (PP) group have vitamin D deffeciency (<20 ng/ml).

Vitamin D levels **(Table 3)** show a significant negative correlation with BMI in (NP, PP and PC)groups. These results agree with Pitt away et al. &Pagliardini et al., who found that body weight was negatively associated with vitamin D status [47,48].

Nora A. found that overweight and obese women had a higher prevalance of vitamin D deficiency compared to those with normal body weight [49]. Also results in **Table(3)** are in line with some studies whish revealed that vitamin D concentration showed strong positive correlation with athero protective lipids (HDL-C) [50-51] while disagree with that of Chiu et al., who showed no relationship between serum levels of 25(OH) D and TG or HDL cholesterol in healthy subjects [52].

Few studies have been carried out on the relationship between serum levels of vitamin D and lipid profiles. a high prevalence of vitamin D deficiency was found in middle‐aged premenopausal Indian women. These results showed inverse correlation of serum 25(OH)D with serum TC, TG, and LDL‐C and a positive correlation with HDL‐C [53].However, no relationship was observed between 25(OH) D and TG or HDL cholesterol [54].

In addition, BMI showed a negative correlation with HDL-C in all studied groups while it correlated differently with other parameters of lipid profile, **Table (4)**.Several data have reported that higher values of BMI associated with a higher plasma triglyceride level, lower HDL cholesterol level, and higher total and non-HDL cholesterol levels[55].

The differences in serumsex hormones and lipid levels even after adjustment for differences in body size were studied. A significant association between endogenous estradiol and HDL-C levels exists in premenopausal women. In addition, the present an inverse correlation between serum estradiol level and BMI[56]. Two roger et al. (2006)suggested two hypotheses to prove the inverse correlation between BMI and estradiol level. First, a high BMI may be associated with ovulatory insufficiency beyond its known role in increasing ovulatory cycles[57].

The higher rate of vitamin D defeciency was found in PP group (64%) in comparison to NC,PC and NP groups (26.6%,6.6% and 36%respectively, **(Table5)**.The differences in the results of vitamin D between the study groups may be affected by many factors: age, weight, and air pollution affected the amount of UVB radiation reaching the earth's surface, lifestyles and therefore skin vitamin D production, [58]. Another factor affecting vitamin status and its reduction in females can be women garments in Islamic territories [59,60].

A new report has shown that exposure to sunlight boosts fertility in both men and women by increasing their levels of vitamin D, a benefit that appears to work on multiple levels. Simple advice for sun exposure and vitamin D3 supplementation can have a profound impact on patient" s health, even if trying to conceive naturally[61].

Table (6) showed a negative corelation (p=0.001) of TNF – α with vitamin D defeciency (<20 ng/ml) in (PP)group while no correlation was found in the other dtudied groups. Previous study explained that spermatozoa may be exposed to pathological concentrations of TNF-α during their passage into the female reproductive tract. Abnormal levels of TNF-α have toxic effect on spermatozoa led to significant loss of their functional and genomic integrity [62].

In humans, vitamin D receptors are present in many female organs, including the ovary, uterus, and placenta. The active form of vitamin D (calcitriol) has many roles in female reproduction. Bound to its receptor, calcitriol is able to control the genes involved in making estrogen [63].

Oral supplementation vitamin D significantly increaseed serum vitamin D levels and insignificantly reduced serum TNF-α level [64]. It is difficult to discern the specific mechanisms by which elevations in systemic 25(OH)D attenuate circulating TNF-α concentrations. Nonetheless, our resaults agree with experimental data showing that vitamin D is inversily associated with TNF-α production [65,66].

Vitamin D influences the functioning of the reproductive system in women and has been associated with PCOS, uterine leiomyomas, endometriosis and *in vitro* fertilization (IVF) outcome. However, further studies on larger groups of patients are needed to establish what role vitamin D plays in the treatment of female infertility [67].

CONCLUSION

Our results found a high rate of vitamin D defeciency in infertile women with positive IgG toxoplasma gondii a compined with significant increase in serum levels of TNF-α. This finding suggests a role for this cytokine in reproductive function and encourages both prompting health education to prevent vitamin D defeciency in female population especially in childbearing age. Further study is needed to explore the relationship between cytokine production with vitamin D defeciency and its assotiation with female infertility.

REFFERENCES

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