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Role of vitamin D, interleukin-17A and interleukin-10 levels in women with recurrent miscarriage

Zeina J. AbduLshaheed
Southern Technical University, College of Health and Medical Technology/Al-Basrah, Iraq.

Ali A. H. Albakaa
Southern Technical University, Al-Nasiriyah Technical Institute, Iraq

Hasan Abd Ali Khudhair
Southern Technical University, Al-Nasiriyah Technical Institute, Iraq
Corresponding author email: zeinajm9@gmail.com

***Abstract*---**Background: RPL is a common early pregnancy complication that is characterized as three or more pregnancy losses occurring before the 20th week of gestation. RPL is a common early pregnancy complication that is characterized as three or more pregnancy losses occurring before the 20th week of gestation. Recurrent miscarriage (RM) is a common occurrence, affecting 15–25% of all pregnancies. The study's goal was to assess vitamin D, interleukin-17a, and interleukin-10 levels in women who had several miscarriages and investigate the relationship between biomarkers to determine the final outcome of pregnancy. Methods: A case control study that included three study groups; thirty five women with RPL, thirty non-aborted pregnant (NAP) women and twenty five apparently healthy women. Patients were selected from those attending Bint Al-Huda Teaching Hospital and Suq Al-Shuyukh General Hospital at Thi-Qar Province (Iraq) during the period between October 2021 and July 2022 after stringent application of the eligibility criteria. Serum samples was collected form each subjects and was evaluated for serum VD, IL-17A and IL-10 levels via enzyme-linked immunosorbent assay (ELISA). Results: The results revealed that serum VD and IL-10 levels in the RPL group were significantly lower than in the healthy control group (HC). The level of IL-10 was considerably lower in the NAP group compared to the HC group, but the level of IL-17A was significantly greater in the RPL group compared to the NAP group. There was a substantial positive link between serum VD and serum IL-17A levels in the RPL and NAP groups, while VD had a significant negative relationship with serum IL-10 in the RPL group, while there

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was a negative correlation in the NAP and HC groups. Conclusions: The combination of VD, IL-17A, and IL-10 might be a potent and cost-effective diagnostic biomarker for RPL. Vitamin D and IL-17A had a substantial positive relationship in the RPL and NAP groups. A significant negative link between VD and IL-10 in the RPL group but a significant positive association in the NAP group, also we found a significant negative correlation between serum IL-17A and IL-10 levels in RPL group, indicating a possible compensating correlation between these biomarkers to determine the final result pregnancy.

***Keywords*---**Recurrent miscarriage, Vitamin D, Interleukin-17A, Interleukin-10, Pregnancy.

Introduction

Miscarriage is the most common pregnancy complication, defined as the termination of a pregnancy before the fetus reaches viability. Despite the fact that only 15% of miscarriages are clinically detected, overall reproductive losses are closer to 50% of conceptions, and hence 1–5% of couples seeking to conceive are affected [1]. It's a rather common event, occurring in 15–25% of all pregnancies and increasing more common as the mother's age increases [2]. Risk factors for the development of RM include chromosome abnormalities, genital structural abnormalities, endocrine disorders, immunological disturbances, viral infection, and prothrombophilia. However, some people have unexplained RM, which might be caused by alloimmunity [3]. Almost half of all RM cases have yet to be resolved [2].

Vitamin D is a secosteroid hormone that regulates bone metabolism and equilibrium [4]. Vitamin D controls the immune system in addition to its physiological function [5], so VD deficiency/insufficiency may raise the risk of numerous chronic diseases requiring immunological disorders. The immune system also plays a role in human reproduction. Vitamin D has been linked to infertility, polycystic ovary syndrome (PCOS),in vitro fertilization(IVF) results, and obstetrical outcomes [6]. Having a low VD status during pregnancy increases the risk of obstetrical problems [7]. Vitamin D insufficiency is thought to affect a large percentage of RM patients[8]. Low levels of VD have also been related to an increased risk of miscarriage during the first trimester[9]. Immunological processes have been hypothesized to explain some occurrences of RPL. However, the specific methods by which the immune system is altered in individuals with idiopathic RSA remain unknown. The identification of a new Th17 subgroup of CD4+ effector T cells, separate from the well-known Th1, Th2, and Treg cells, has significantly expanded our understanding of T cell-mediated immunity [10and11]. In unexplained recurrent spontaneous abortion (URSA) patients, a Th17/Treg imbalance has also been found [12]. Additionally, it has been proposed that pro-inflammatory Th17 type cytokines that promote allograft rejection may jeopardize pregnancy, whereas regulatory T-cell related cytokines that promote fetal allograft tolerance may improve pregnancy outcomes [13and 14]. To mediate inflammation, autoimmunity, and immune rejection of foreign tissue, Th17 cells release the hallmark cytokine IL-17A. The retinoid orphan nuclear receptor (RORC) is

recognized to be a critical regulator of human Th17 cell lineage development[15and16]. Interleukin-10 is an immunosuppressive cytokine that is required during pregnancy to maintain the balance of pro-inflammatory and anti-inflammatory effectors. It shields the fetus from the maternal immune reaction by reducing Th1-cell activity and the generation of TNF-α and IFN-γ [17]. The purpose of this study is to look at the levels of VD, IL-17A, and IL-10 in women who had multiple miscarriages, as well as the relationship between these biomarkers to determine the final outcome of pregnancy and to test the hypothesis that they could be used as supplement therapy.

Materials and Methods

Subjects and study design

A case control study with three study groups: thirty-five women with RPL (unexplained consecutive pregnancy loss), thirty NAP women (control group), and twenty-five apparently healthy women (HC). All research groups ranged in age from 22 to 34 years old. Patients were chosen among those attending Bint Al-Huda Teaching Hospital and Suq Al-Shuyukh General Hospital (especially the women's advisory) in Thi-Qar Province (Iraq) between October 2021 and July 2022 after strict adherence of the qualifying criteria outlined below. To fulfill international ethical standards for research, each woman participated in the recent study provided written consent, and the present research was authorized by the ethical consideration committee at Southern Technical University, Al-Nasiriyah Technical Institute. Suq Al-Shuyukh General Hospital performed laboratory tests for serum VD, IL-17A, and IL-10.

Eligibility criteria

For RPL group, women who had any of the following criteria were excluded from the current study: women with uterine, inherited, infectious, endocrine or autoimmune diseases which commonly cause RPL, patients with anti-phospholipid syndrome, underwent corticosteroid therapy (for the last 4 weeks) or taking any biological agent, recent surgery (during the last 4 weeks) and recent blood transfusions (during the last 6 months), whereas the women with following criteria were included; pre-menopausal females (18-44) years of age at consent, trying to conceive, documented history of unexplained RPL, non-smokers and had none of the above mentioned exclusion criteria. For NAP group, the women with following criteria were included in this group; had experienced one or two pregnancies with natural labor before enrollment, with no history of miscarriage, had no history of any of common conditions that associated with RPL, not taking corticosteroid therapy for the last 4 weeks, absence of any autoimmune or chronic diseases, absence recent surgery and blood transfusions (during the last 6 months), not taking any biological agents, had matched age and body mass index (BMI) with the RPL women and non-smoker. The third study group (HC) inclusion criteria were the same inclusion criteria of the NAP women and in addition, subjects with even a simple infection were also excluded. All women of this group had no pregnancy at time of samples collection.

Samples collection

By vein puncture, 4–5 milliliters (ml) of peripheral blood were obtained from each individual and placed in a vacuum gel tube. At room temperature, the obtained blood samples were allowed to complete the clotting process. The sera samples were collected after the blood samples were separated using a centrifuge (Hettich, Germany) at 3000 g. The separated sera samples were kept at -20 degrees Celsius (oC) until they were needed for serological testing.

Determination of vitamin D concentration

Serum VD was measured Total 25-OH Vitamin D ELISA Kit (Demeditec, Germany). Immunoenzymetric assay for the quantitative determination of 25-hydroxyvitamin D2 and D3 (25OH-D2 and 25OH-D3) in serum.

Determination of IL-17A concentration

Human vitamin E ELISA Kit was used to assess serum IL-17A. (Shanghai YL Biont, China). This kit assays human IL-17A using ELISA based on biotin double antibodies (Abs) sandwich technique. Incubate IL-17A in wells that have been pre-coated with IL-17A monoclonal Abs. Then, combine anti-IL-17A Abs tagged with biotin with streptavidin- horseradish peroxidase to generate an immunological complex. After incubation and washing, remove any unbound enzymes. Combine substrates A and B. The solution will then become blue and then yellow due to the acidic action. The hues of the solution and the concentration of human IL-17A are connected.

Determination of IL-10 concentration

Serum Interleukin-10 was measured ELISA Kit (Demeditec, Germany). Immunoenzymetric assay for the quantitative determination of IL-10 in serum.

Statistical analysis

Data were transformed into a computerized database structure using Microsoft Office Excel 2010 software. The statistical analysis was computer-assisted using the Statistical Package for Social Sciences (SPSS) software version 27. The frequency distribution and percentages were calculated initially, followed by the Chi-Square statistical test, simple correlation (r), and simple liner regression to test for relationships between variables. A statistically significant p-value <0.05 was considered.

Results

In the current study, 90 women were enrolled: 35 with RPL (unexplained successive pregnancy loss), 30 with NAP, and 25 with HC. The research subjects' ages ranged from 22 to 34 years old, with matching BMI (Table 1).

Table (1): Study groups categorization

Total Subjects (n=90)		
Recurrent Pregnancy Loss	Non Aborted Pregnant	Healthy Control
n=35	n=30	n=25

n: number.

Figure 1 depicts the Vitamin D values across all research groups. The frequency percent of the lowest level of VD was considerably (P<0.05) greater in RPL group 18 (51.4%) than in NAP 1(3.30%). In terms of mean titer, RPL had the lowest mean titer (19.74 ng/ml) when compared to the NAP group (23.69 ng/ml) and the HC group (25.48ng/ml) with a significant differences (p<0.05).

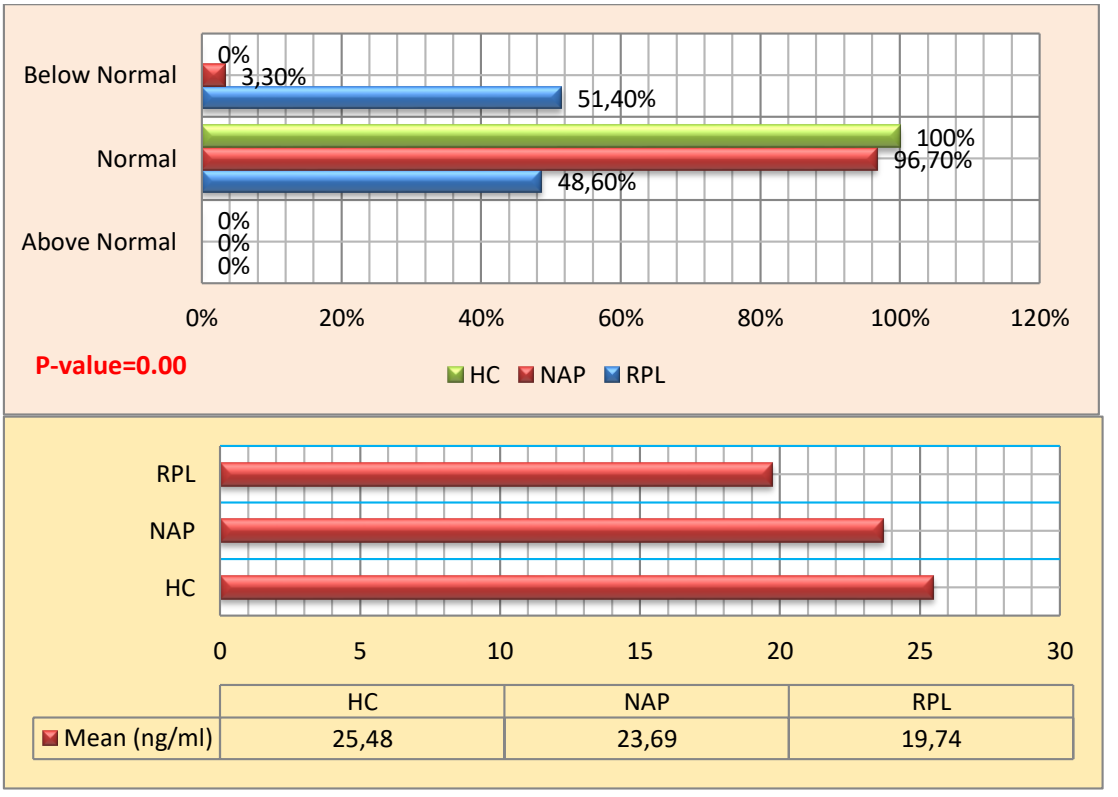

Figure (1): The results of frequency (%) and mean titer of vitamin D in all study groups(ng: nanogram , ml: milliliter, below normal:>20 , normal (20-40) and above normal:<40).

Figure (2) demonstrates that the great majority of the NAP group 19(63.3%) had serum interleukin-17A levels below normal compared to the RPL group 18(48.6%) and the HC group 1(4%), with a significant difference (p<0.05). For mean titer Interleukin-17A titers (Figure 2), the NAP group had the lowest (31.05 ng/l), followed by the RPL group (40 ng/l) and the HC group (123.46 ng/l), with significant differences (p<0.05).

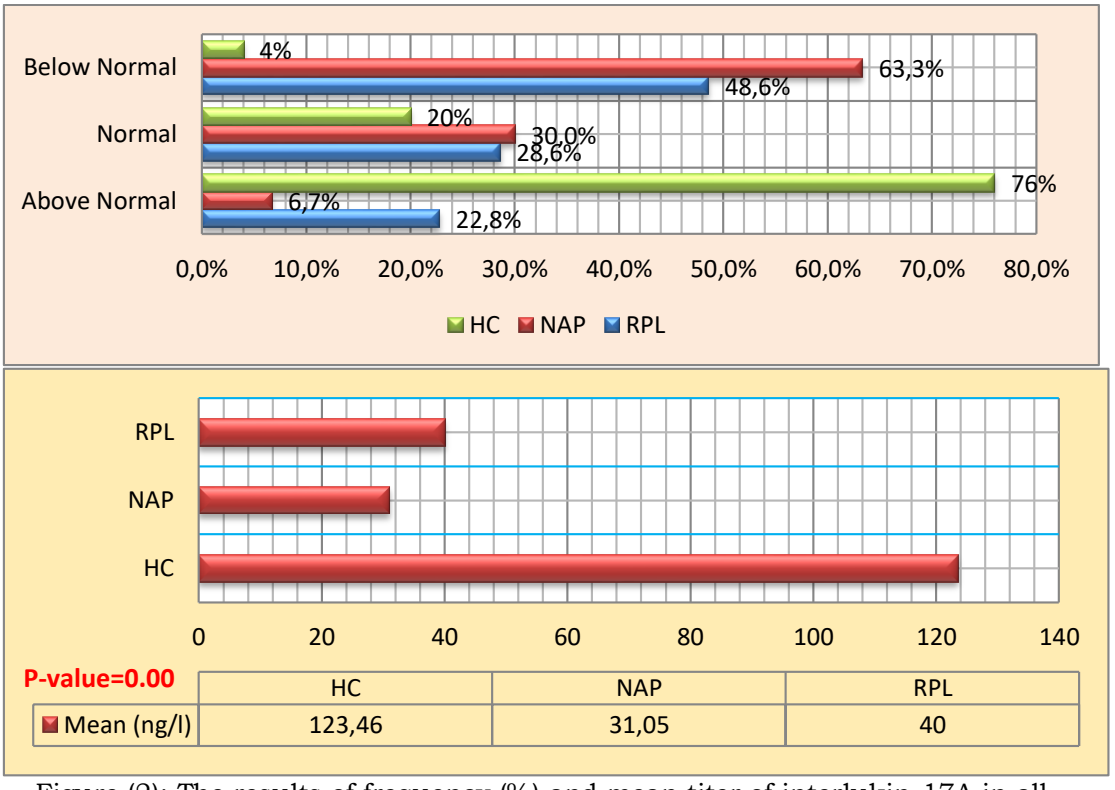

Figure (2): The results of frequency (%) and mean titer of interlukin-17A in all study groups (Below normal: >25, normal: (25-50), above normal : <50, ng: nanogram and L: liter).

The serum interleukin-10 levels in patients and control groups are compared in Figure (3). The frequency of interleukin-10 below normal level was high in the NAP group 28(93.3%) followed by HC group 20(80%) and RPL group 27(77.1%). With no Significant difference at *(P*>0.05). For mean titer, RPL group exhibited the highest mean titer (12.7 pg/ml) compared with HC group (3.33 pg/ml) and NAP group (2.4 pg/ml). The difference in mean titers between all groups was significant (p<0.05).

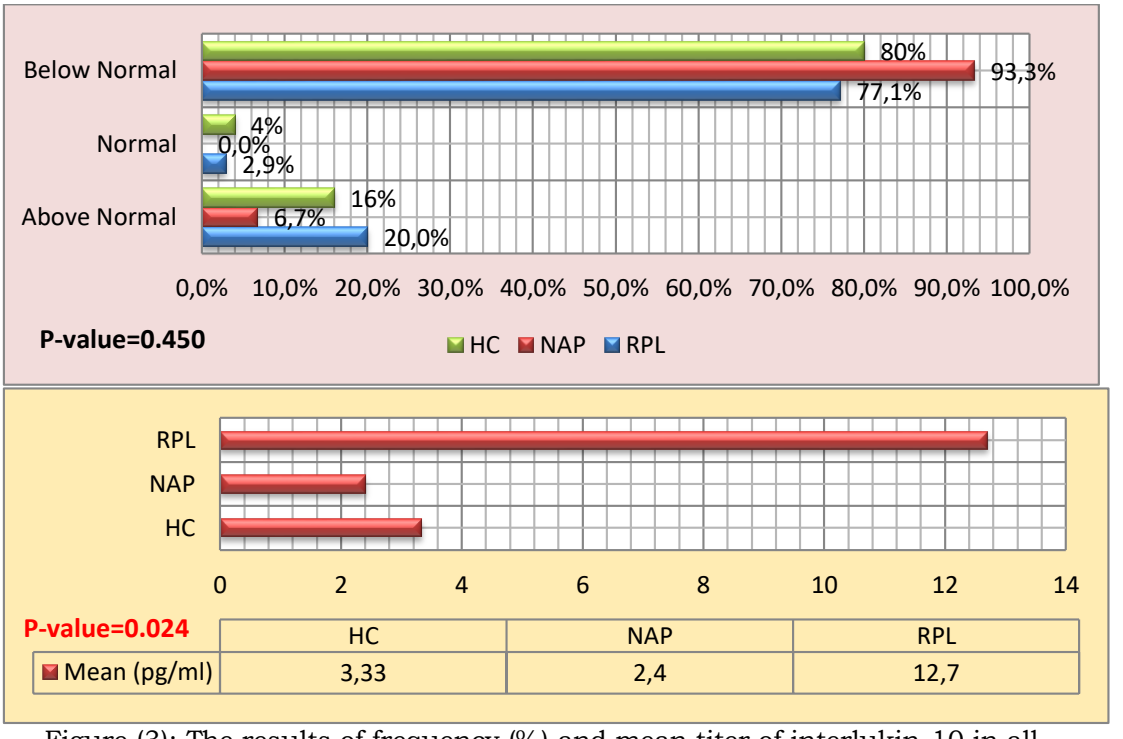

Figure (3): The results of frequency (%) and mean titer of interlukin-10 in all study groups (below normal :>5, normal: (5-10), above normal: <10 , Pg : pictograms and ml: milliliter).

The results of the correlation between VD and IL-17A in all study groups are demonstrated in Table **(2)**. Among 18/35 women with below normal VD level of RPL group, 1/18(5.6%) was with above normal IL-17A frequency %. On the other hand, among 17/35 women with normal VD level,7/17(41.2%) were with above normal IL-17A frequency %. The total mean titer of serum IL-17A was lower (24.3ng/l) compared to women with normal VD level (56.5ng/l) respectively (p<0.05). For NAP group, among 1/30 women with below normal VD level, 1/1(100%) were with below normal IL-17A frequency %. On the other hand, among 29/30 women with normal VD level, 18/29 (62.1%) were with below normal IL1-7A frequency %. The differences in IL-17A frequency % between the women with below normal and normal VD were significant (p<0.05). For mean titer, among women with below normal VD level the total mean titer of serum IL-17A was lower (21.4ng/l) compared to women with normal VD level (31.4ng/l) respectively (p<0.05). In RPL and NAP groups, the results of regression analysis (Figure **4**) showed a positive significant (p<0.05) correlation between serum VD and serum IL-17A.

Table (3.5): Correlation between vitamin D and interleukin-17A in all study groups

Parameters			Interleukin-17A (ng/l)								p. value
			Below N (<25)		Normal (25-50)		Above N (>50)		Total		
			FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	
Vitamin D (ng/ml)	RPL (n=35)	Below N (n=18)	9(50)	15.3	8(44.4)	31.2	1(5.6)	50.6	18(100)	24.3	<0.05
		Normal (n=17)	8(47.1)	18.5	2(11.7)	26.4	7(41.2)	108.6	17(100)	56.5	
		Total (n=35)	17(48.6)	16.9	10(28.6)	28.8	8(22.8)	101.3	35(100)	40	
	NAP (n=30)	Below N (n=1)	1(100)	21.4	0(0)	0	0(0)	0	1(100)	21.4	<0.05
		Normal (n=29)	18(62.1)	22.3	9(31)	30.9	2(6.9)	114.5	29(100)	31.4	
		Total (n=30)	19(63.3)	21.8	9(30)	30.9	2(6.7)	114.5	30(100)	31.0	
	HC (n=25)	Below N (n=0)	0(0)	0	0(0)	0	0(0)	0	0(0)	0	-------
		Normal (n=25)	1(4)	24.8	5(20)	31.6	19(76)	152.8	25(100)	123.4	
		Total (n=25)	1(4)	24.8	5(20)	31.6	19(76)	152.8	25(100)	123.4	

RPL: recurrent pregnancy loss, NAP: non-aborted pregnant, HC: healthy control, n:number , N: normal ,FR (%): frequency (percent), ng: nanogram, L: liter and ml: milliliter

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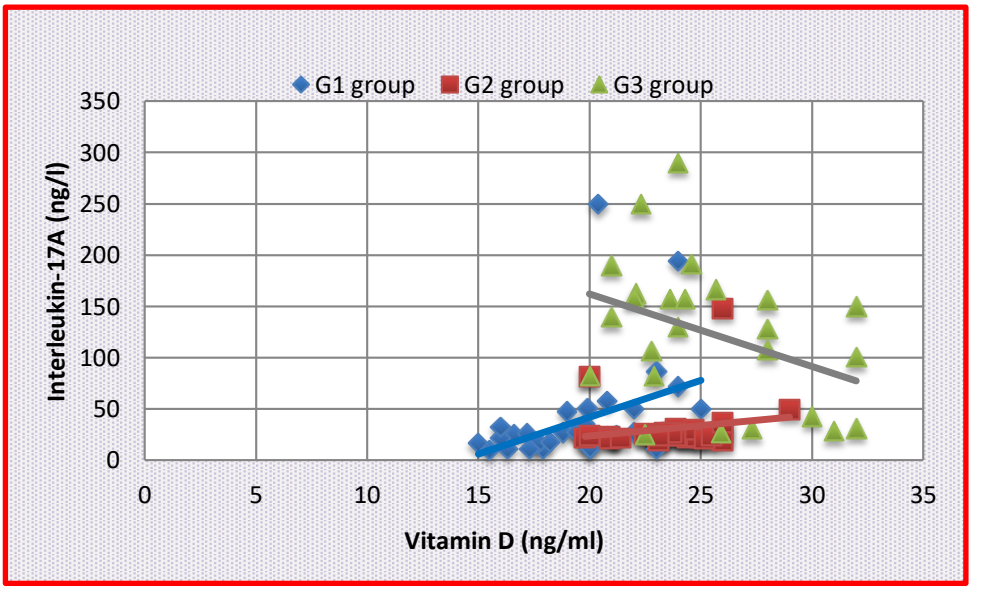

Figure (3.9): Regression analysis of vitamin D and interleukin-17A in all study groups (G1: recurrent pregnancy loss, G2: non-aborted pregnant, G3: healthy control, ng: nanogram , L: liter, nmol: nanomole).

In Table 3, the correlation between VD and IL-10 in all study groups is illustrated. For RPL and NAP groups, the frequency % of below normal IL-10 was lower with significant difference (p<0.05) in the women with below normal VD level 13/18(72.2%) and 1/1(100 %) respectively, when compared with women with normal VD level 14/17(82.4%) and 27/29(93.1%) respectively. The results of IL-

10 mean titer were in a similar profile to frequency %, mean titer was (13.6 pg/ml) and (0.9 pg/ml) respectively among women with below normal VD with significant difference (p<0.05), in comparison to women with normal VD level (11.7 pg/ml) and (2.8 pg/ml) respectively. The results of the present study (Figures **5**) identified a negative correlation (p<0.05) between serum VD and serum IL-10 levels in RPL group, while the results of NAP groups in the same figure showed a positive correlation, with a significant difference (p<0.05).

Table (3): Correlation between vitamin D and interleukin-10 in all study groups

Parameters			Interleukin-10 (pg/ml)								p. value
			Below N (<5)		Normal (5-10)		Above N (>10)		Total		
			FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	
Vitamin D (ng/ml)	RPL (n=35)	Below N (n=18)	13(72.2)	1.0	1(5.6)	5.3	4(22.2)	37.7	18(100)	13.6	<0.05
		Normal (n=17)	14(82.4)	0.9	0	0	3(17.6)	61.9	17(100)	11.7	
		Total (n=35)	27(77.1)	1.0	1(2.9)	5.3	7(20)	49.8	35(100)	12.7	
	NAP (n=30)	Below N (n=1)	1(100)	0.9	0(0)	0	0(0)	0	1(100)	0.9	<0.05
		Normal (n=29)	27(93.1)	1.2	0(0)	0	2(6.9)	19.6	29(100)	2.8	
		Total (n=30)	28(93.3)	1.0	0(0)	0	2(6.7)	19.6	30(100)	2.4	
	HC (n=25)	Below N (n=0)	0(0)	0	0(0)	0	0(0)	0	0(0)	0	-------
		Normal (n=25)	20(80)	0.9	1(4)	7.2	4(16)	14.6	25(100)	3.3	
		Total (n=25)	20(80)	0.9	1(4)	7.2	4(16)	14.6	25(100)	3.3	

RPL: recurrent pregnancy loss, NAP: non-aborted pregnant, HC: healthy control, n:number, FR (%): frequency (percent),N: normal, ng: nanogram, pg: picograms and ml: milliliter

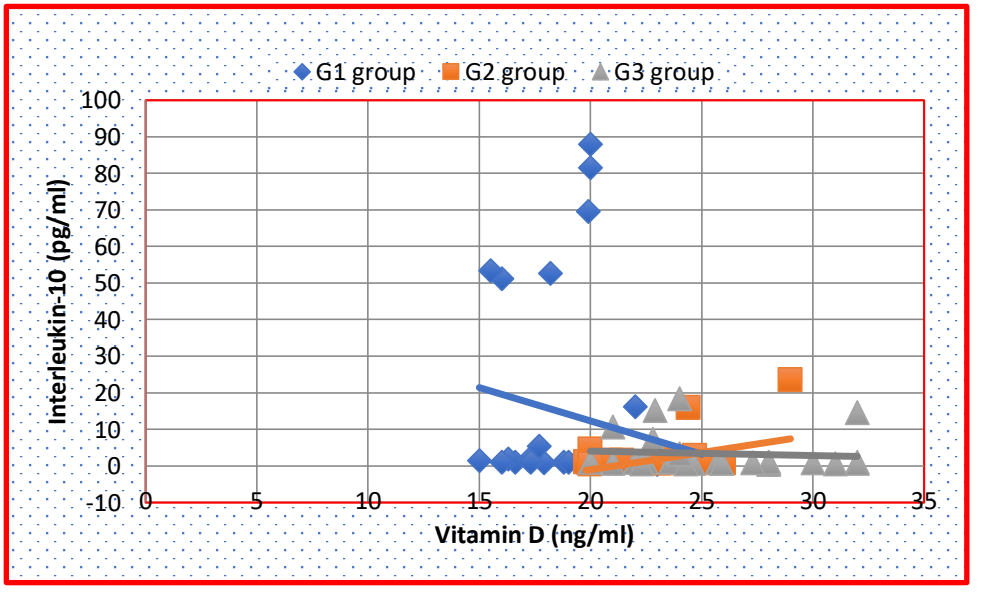

Figure (5): Regression analysis of vitamin D and interleukin-10 in all study groups (G1: recurrent pregnancy loss, G2: non-aborted pregnant, G3: healthy control, ng: nanogram , ml: milliliter, pg: pictograms).

In Table **(4),** the correlation between IL-17A and IL-10 in all study groups is illustrated. For RPL and HC groups, the frequency % of below normal IL-10 was lower with significant difference (p<0.05**)** in the women with below normal IL-17A level 11/17(64.7%) and 1/1(100%) respectively when compared with subjects with normal IL-17Alevel 10/10(100%) and women with above normal IL-17A level 15/19(78.9%) respectively. The results of IL-10 mean titer were in a similar profile to frequency%, mean titer was higher (20.1pg/ml) and (0.9pg/ml) respectively among subjects with below normal IL-17A with significant difference (p<0.05) in comparison to subjects with normal IL-17A level (11.4 pg/ml) and comparison to subjects with above normal IL-17A (3.4 pg/ml) respectively. The results of the present study (Figures **6**) identified a negative correlation between serum IL-17A and serum IL-10 levels in RPL group (p<0.05). while the results of NAP and HC groups in the same figure showed a positive correlation (P<0.05) for HC group and NAP group(p>0.05).

Table (4): Correlation between interleukin-17A and interleukin-10 in all study groups

Parameters			Interleukin-10 (pg/ml)								p. value
			Below N (<5)		Normal (5-10)		Above N (>10)		Total		
			FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	
Interleukin-17A (ng/l)	RPL (n=35)	Below N (n=17)	11(64.7)	1.0	1(5.9)	5.3	5(29.4)	65.3	17(100)	20.1	<0.05
		Normal (n=10)	10(100)	1.0	0(0)	0	0(0)	0	10(100)	1.0	
		Above N (n=8)	6(75)	0.9	0(0)	0	2(25)	42.9	8(100)	11.4	
		Total (n=35)	27(77.1)	1.0	1(2.9)	5.3	7(20)	54.1	35(100)	12.7	
	NAP (n=30)	Below N (n=19)	18(94.7)	1.2	0(0)	0	1(5.3)	15.8	19(100)	2.0	>0.05
		Normal (n=9)	8(88.9)	1.1	0(0)	0	1(11.1)	23.5	9(100)	3.5	
		Above N (n=2)	2(100)	0.6	0(0)	0	0(0)	0	2(100)	0.6	
		Total (n=30)	28(93.3)	0.7	0(0)	0	2(6.7)	19.6	30(0)	2.4	
	HC (n=25)	Below N (n=1)	1(100)	0.9	0(0)	0	0(0)	0	1(100)	0.9	<0.05
		Normal (n=5)	4(80)	0.7	0(0)	0	1(20)	14.6	5(100)	3.4	
		Above N (n=19)	15(78.9)	0.9	1(5.3)	7.2	3(15.8)	14.6	19(100)	3.4	
		Total (n=25)	20(80)	0.8	1(4)	7.2	4(16)	14.6	25(100)	3.3	

RPL: recurrent pregnancy loss, NAP: non-aborted pregnant , HC: healthy control, n:number ,FR (%): frequency (percent), N:normal , ng: nanogram, pg: picograms , L: liter and ml: milliliter

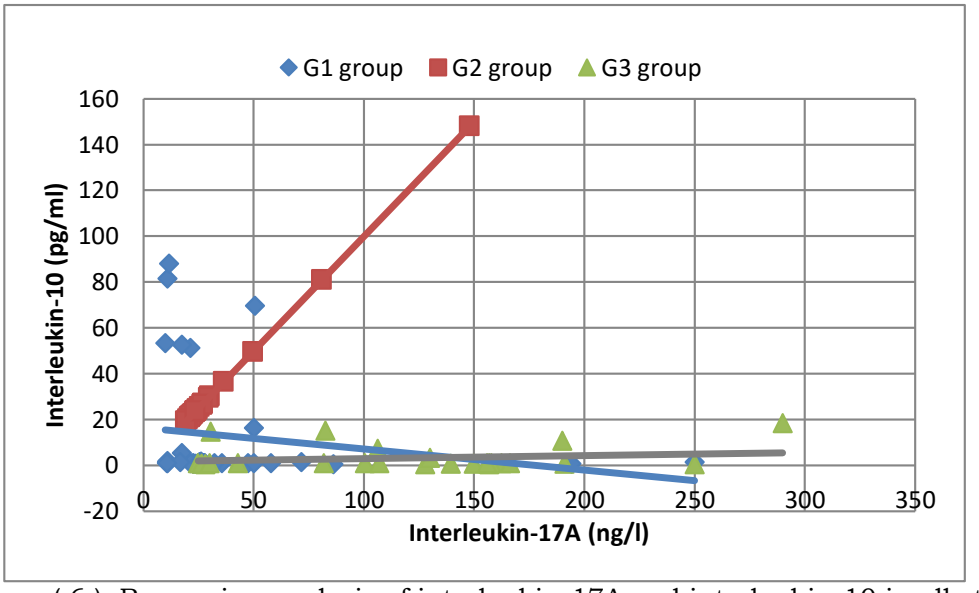

Figure (6): Regression analysis of interleukin-17A and interleukin-10 in all study groups(G1: recurrent pregnancy loss, G2: non-aborted pregnant , G3: healthy control, ng: nanogram, pg: picograms, L: liter and ml: milliliter).

Discussion

Recurrent spontaneous miscarriage, also known as RM, habitual abortion, or RPL, is defined as more than three consecutive miscarriages prior to 20 gestational weeks. Recurrent pregnancy loss affects 15% of pregnant women. Because the cause of RPL is unknown, ongoing clinical and laboratory investigations are required[18]. Previous research has found that chromosome abnormalities, endocrine diseases, uterine abnormalities, placental anomalies, hormonal problems, thrombophilia, infections, nutritional disorders, autoimmune disease, and anatomy are all involved in certain RPL cases [19 and 20].

Hypovitaminosis D is a common health issue among pregnant women. The RPL group in the current study had a low level of VD. Vitamin D levels were higher in the NAP and HC groups, which could be attributed to VD supplementation protocol during pregnancy, as VD is known to be a risk factor for RM. This was similar to an earlier Iranian study on pregnant women [21]. Pregnancy complications can be increased by having a low VD status [7]. According to findings, a high proportion of RM patients have VD deficiency [8]. Low VD levels have also been linked to an increased risk of miscarriage in the first trimester [9]. Given the variety of factors that influence VD levels in different countries, such as sunlight exposure, clothing habits, diet, and study season, variation in VD deficiency prevalence is to be expected. However, our study location (Thi-Qar province) is sunny most of the time, and living in an apartment is not as common as it is in other countries. So, perhaps the most influencing factors that determine VD levels in women are clothing habits, harmful effects of the sun on the skin, and ignorance of the necessity of sunlight for VD synthesizing. Vitamin D is an important regulator of systemic inflammation and oxidative strees (OS). In

turn, VD molecular and cellular actions slow down OS, cell, and tissue damage. Hypovitaminosis D, on the other hand, impairs mitochondrial function while increasing OS and systemic inflammation [22]. The placenta, specifically the maternal decidua, which forms the interface with newly implanted fetal placental trophoblast cells, is a site of immune privilege that may act as an important target for VD. It has previously been proposed that VD may aid in successful implantation by decreasing decidual T-cell function [23]. Vitamin D specifically targets Th1 and Th2 cells. The mechanism of its activity is not fully understood, but it is thought to suppress the production of Th1 cytokines such as IL-2, IFN-, IL-1, IL-6, and IL-8 while increasing the production of Th2 associated cytokines[24].

Interleukin-17A is produced by a variety of adaptive and innate immune cells, including T helper 17 (Th17) cells, CD8 + T cells, natural killer T cells (NKT), and innate lymphoid cells [25]. In our present study, we found lowest frequency of below normal IL-17A levels in RPL women compared to NAP women, which is consistent with another study[14]. that found non-pregnant women with unexplained RPL had higher Th17 cell numbers in circulating blood than parous controls. Furthermore, Th17 cells were shown to be elevated in the deciduas of unavoidable abortions, such as the progression stage of abortion [26]. As a result, RPL is linked with a Th17 cell-mediated inflammatory immunological response in addition to dominating Th1 immunity [27]. According to certain studies, the frequency of Treg cells decreases in the peripheral blood and decidua of women with RPL, but Th17 cells often rise in these individuals. Treg cells control Th17's activity as an effector cell. The balance of Th17 and Treg cells is required to maintain implantation and hence a healthy pregnancy [28]. An increase in Th17a cells vs a reduction in Treg cells implies an immune imbalance that leads to pregnancy failure [29]. Our study also found that the level of IL-17a was lower among pregnant women with no history of abortion. According to recent research, the number of Th17 cells in the peripheral blood and decidua is lower in pregnant women with URSA compared to parous controls [30 and14]. Furthermore, Wang et al. demonstrated that Th17 cells were abundant in URSA patients' peripheral blood and decidua, and that there was an inverse association between Th17 cells and Treg cells. In our study, we discovered that the HC group had the largest proportion of IL-17a levels that were above normal for unexplained reasons. This study's findings (Table 2.) revealed a considerable increase in IL-17A among individuals with below-normal VD levels compared to depletion levels among members with normal VD levels, and the two biomarkers were positively related in RPL (Figure 4). A favorable connection between two biomarkers was also seen in the NAP group. As a result, we hypothesize that the positive connection between VD and IL-17A is related to vitamin D's present inability to regulate Treg/Th17 balance in RPL patients. Additionally, vitamin D supplementation is required to maintain appropriate immunity for a healthy pregnancy[8].

Interleukin-10 is a pleiotropic cytokine generated by activated Th2 cells, B-cells, monocytes, and macrophages that plays an important role in maintaining maternal–fetal tolerance [31and 32]. In the current study, showed the level of IL-10 was low in the RPL group of women. The data suggest that IL-10 has a beneficial function in the prevention of spontaneous abortion (SAb); when IL-10

levels rise, the risk of SAb reduces. IL-10 has been shown to be a powerful defender against vascular dysfunction, and IL-10 augmentation can operate as an immunotherapeutic intervention to regulate hostile pregnancy outcomes[17]. Investigated the role of progesterone in cytokine production According to them, progesterone promotes the growth of Th cells that produce Th2-type cytokines such as IL-10. They went on to speculate that progesterone synthesis at the placental level may be responsible, at least in part, for the increase in Th2-type cytokine production[33]. Recently, [34]. demonstrated that IL-10 may have a role in term delivery regulation. My study also found that the level of IL-10 fell in the NAP group, these result disagreement with previous research that found that healthy pregnancy is associated with higher levels of IL-10, but pathologic pregnancies are associated with decreasing levels of IL-10 [35].

According to the findings of our investigation, reduced IL-10 production may result in pregnancy loss. This shows that IL-10 is important throughout the first few months of pregnancy. These findings raise the prospect of IL-10 based treatment becoming a reality for perplexing immunological pregnancy illnesses [36]. Several studies have shown that IL-10 plays a vital role in pregnancy maintenance because its expression is increased in normal pregnancy but is disrupted during premature labor. Women who were threatened with abortion had considerably lower blood concentrations of anti-inflammatory cytokines and greater levels of pro-inflammatory cytokines compared to healthy controls[37] . Consistent with these studies, the current study found (Table 3) a significant decrease in IL-10 levels among women with below normal VD levels compared to women with normal VD levels, and the IL-10 level was negatively correlated with VD level in RPL group and positive correlated with VD level in NAP group (Figure 5).

The current study findings also showed lower levels of serum IL-10 among subjects with below normal IL-17A levels in RPL group (Table4) and there were significant negative correlation between both biomarkers within the last mention group while positive correlation between both biomarkers within in NAP and HC groups (Figure 6). There was a significant association between the number of IL-17-producing T cells and the ratios of TNF-/IL-10 and IFN—/IL-10-producing CD3+CD4+ cells. We also discovered a strong positive connection between the IL-17+ T/Treg cell ratio and type 1 cytokine production[38] . This corresponds to the activation of Th17 and Th1 immune response, which is consistent with our findings. Thus, the findings imply that an imbalance between Th1 and Th2 cells, as well as the extra biological consequences of elevated IL-17+ T cell counts, may cause an inflammatory immune response that leads to the development of RPL.

The present study's main limitation was the small sample size. The second constraint was the actual sensitivity and specificity of the biomarker measurement kits utilized in patient samples. Third, the patients' selection criteria (exclusion and inclusion) had a significant impact on the study's sample size, owing to the difficulty in finding patients with RPL who satisfied the requirements.

Conclusion

Vitamin D and IL-10 levels were much lowest in RPL women, while IL-17A was highest in RPL therefore the combination between them could be a powerful and cost-effective diagnostic biomarkers for RPL. A significant positive association of VD and IL-17A in RPL and NAP groups while significant negative association of VD and IL-10 in RPL group. Also we found negative association between IL-17a and IL-10 levels in RPL group.

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List of Abbreviates:

- **Abs:** antibodies.
- **IVF:** in vitro fertilization.
- **PCOS:** polycystic ovary syndrome .
- **RORC:** retinoid orphan nuclear receptor **.**
- **URSA:** unexplained recurrent spontaneous abortion.
-**BMI:** body mass index.
-**ELISA**: enzyme- linked immunosorbent assay.
-**FR(%):** frequency.
-**HC:** healthy control**.**
-**IL-10:** interleukin-10.
-**IL-17A:** interleukin-17a.
-**L:** liter.
-**ml:** milliliter.
-**NAP:** non-aborted pregnant **.**
-**ng :** nanogram.
-**ºC:** Celsius degree.
-**OS:** oxidative stress.
-**pg:** pictogram.
-**RM:** recurrent miscarriage.
-**RPL:** recurrent pregnancy loss**.**
-**Sab:** spontaneous abortion.
-**VD:** vitamin D.

Figures Legends

- **Figure (1):** The results of frequency (%) and mean titer of vitamin D in all study.
- **Figure (2):** The results of frequency (%) and mean titer of interleukin-17A in all study.
- **Figure (3):** The results of frequency (%) and mean titer of interleukin-10 in all study.
- **Figure (4):** Regression analysis of vitamin D and interleukin-17A in all study groups.
- **Figure (5):** Regression analysis of vitamin D and interleukin-10 in all study groups.
- **Figure (6):** Regression analysis of interleukin-17A and interleukin-10 in all study groups.

Table Legends

- **Table (1):** Classification of the study groups.
- **Table (2):** Correlation between vitamin D and interleukin-17A in all study groups.
- **Table (3):** Correlation between vitamin D and interleukin-10 in all study groups
- **Table (4):** Correlation between Interleukin-17A and Interleukin-10 in all study groups.