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Evaluation of Selenium and Vitamin E Levels in Women with Recurrent Miscarriage

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***Abstract*— The most frequent pregnancy issue is a miscarriage, which is defined as the spontaneous end of pregnancy before the fetus reaches viability. The current research aims to identify the potential significance of selenium (Se) and vitamin E (VE) as predictive diagnostic biomarkers in recurrent pregnancy loss (RPL).**

Methods: A case control study that included three study groups; 35 women with RPL, 30 non-aborted pregnant (NAP) women, and 25 healthy women. After rigorously applying the eligibility requirements, patients were chosen from those visiting Bint Al-Huda Teaching Hospital and Suq Al-Shuyukh General Hospital in Thi-Qar Province (Iraq). Serum VE and serum Se levels were determined using the ELISA and atomic absorption spectrophotometer techniques, respectively.

Results: The outcomes showed that the RPL group's serum VE and Se below normal levels were (88.6% and 62.9% respectively) lower than in the healthy control group (28% and 24% respectively). The level of VE was depleted in NAP group (93.3%) compared to HC group (28%) with significant differences, whereas the level of Se was significantly lower in RPL group (62.9%) compared NAP group (13.3%).

Conclusions: A significant positive association of VE and Se in RPL and a significant negative association of VE and Se in NAP and HC.

Keywords*— *Recurrent miscarriage, Selenium, Vitamin E, Pregnancy.

I. INTRODUCTION

The most frequent pregnancy problem is a miscarriage, which is defined as the end of a pregnancy before the fetus reaches viability. Even though only 15% of miscarriages are clinically identified, total reproductive losses are closer to 50% of conceptions, and thus problem affects 1-5% of couples attempting to conceive [1]. It's a rather typical occurrence, occurring in 15-25% of all pregnancies and becoming more likely as the mother becomes older [2]. Chromosome abnormalities, genital structural abnormalities, endocrine disturbances, immunological disturbances, viral infection, and pro-thrombophilia are all risk factors for the development of RM. However, some individuals have inexplicable RM, which may be due to all immunity [3]. Vitamin E has long been recognized to be an important antioxidant in the body. However, it was not until the late 1980s and early 1990s that its activities as a regulatory molecule were hypothesized [4]. In 1922, Evans and Bishop discovered VE, which was first described as an "anti-sterility factor X" that was essential for reproduction [5]. Vitamin E is a fat-soluble vitamin required by higher animals that functions as an antioxidant for lipids [6]. Female infertility, miscarriage, premature delivery, eclampsia, and other pregnancy-related disorders may be occurring due to VE deficiency [7 and 8].

Selenium is a trace element that acts as a cofactor for numerous enzymes in the human body and has antioxidant activity that participates in the synthesis of seleno-proteins [9]. Seleno-proteins perform a variety of functions, including; thyroid homeostasis, reproductive enhancement, and immune/antioxidant function optimization [10].

Infertility, miscarriage, and retained placenta are all consequences of Se insufficiency in women [11]. Selenium deficiency may be associated with pregnancy complications، preeclampsia, premature delivery, fetal growth retardation, and low birth weight [12] and the Se needs increase during pregnancy and nursing due to Se transfer to the fetus through the placenta and to the newborn via breastfeeding, respectively [13]. Thus, the present study aims are to detect the possible role of Se and VE in RPL as predictive diagnostic biomarkers and elucidate the relationship between the two biomarkers to determine the outcome of the pregnancy and to hypothesize to use them as supplement therapy.

II. *Methodology*

*A. M*ethodology

1. **Subjects and study design:** A case-control research that involved three study groups: 35 women with RPL

(unexplained successive pregnancy loss), 30 NAP women (HC), and 25 apparently healthy women (HC). The age of all study groups was 22 to 34 years. Patients were selected from those attending Bint Al-Huda Teaching Hospital and Suq Al-Shuyukh General Hospital (particularly, the women's advisory) in Thi-Qar Province (Iraq) during the period between October 2021 and July 2022 after stringent application of the eligibility criteria mentioned below. Written approval was taken from each woman enrolled in the recent study to meet the international ethical standards for research, and the current research was approved by the ethical consideration committee at Southern Technical University, Al-Nasiriyah Technical Institute. Laboratory tests for serum Se have been done at the University of Thi-Qar, College of Science, Department of Chemistry, whereas serum VE test was performed at Suq Al-Shuyukh General Hospital.

2. **Eligibility criteria:** Women in the RPL group who met any of the following criteria were excluded from the current study: Women with uterine, genetic, viral, endocrine, or autoimmune illnesses that usually induce RPL, patients with anti-phospholipid syndrome had recently received blood transfusions (during the last 6 months), corticosteroid therapy (for the last 4 weeks) or taking any biological agent and recent surgery (during the last 4 weeks In contrast, women who met the following criteria premenopausal females (18–44) years old at consent, trying to get pregnant, having a history of unexplained RPL, not smoking, and not meeting any of the exclusion criteria listed above were included in the study Women who met the following requirements were put into the NAP group: they had one or two normal labor pregnancies prior to enrollment, no history of loss, no history of any of the main disorders linked to RPL, weren't taking corticosteroid therapy for the last 4 weeks, never had recent surgery or blood transfusions (within the previous 6 months), weren't taking any biological agents, and had matched age and body mass index (BMI) with the RPL women and non-smoker. The inclusion criteria for the third research group (HC) were identical to those for the NAP women, and moreover, participants who even had a mild infection were rejected. None of the women in this group were pregnant.
3. **Samples collection:** From each subject, 4–5 milliliters (ml) of peripheral blood were collected within a vacuum gel tube by way of vein puncture. The acquired blood samples were allowed to finish clotting at room temperature. After separating the blood samples using a centrifuge (Hettich, Germany) at 3000 g, the sera samples were then extracted. Until they were required for serological testing, the separated sera samples were kept at a temperature of -20 Celsius (^{o}C).
4. **Determination of vitamin E concentration:** Serum VE was measured by using human vitamin E ELISA Kit (Shanghai YL Biont, China). To measure human VE, this kit uses ELISA based on the biotin double antibodies (Abs) sandwich technique. Incubate VE in wells that have been pre-coated with VE monoclonal Abs. Then, add biotin-labeled anti-VE Abs to generate an immunological complex with streptavidin-horseradish peroxidase. 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 colors of the solution and the concentration of human VE are associated in a beneficial way.
5. **Determination of selenium concentration:** The sera samples were digested by adding 2 ml of concentrated nitric acid (BDH, England) and 1 mL of concentrated per. Chloric acid (BDH, England) to 0.5 ml of subject serum in a Pyrex tube. The mixture was heated for 1 hour at 160 ˚C using paraffin oil (BDH, England) bath, then samples were cooled, and the volume was completed to 10 ml by 0.3 N of hydrochloric acid (BDH, England). Then the digested sera were used to measure the serum Se level by the atomic absorption spectrophotometer technique using Flame Atomic Absorption Device (Buck Scientific Model 210 VGP, England). In this technique, in a lean air-acetylene flame, light from a hollow cathode lamp was absorbed by ground state atoms. The amount of light absorbed was proportional to the concentration of gaseous atoms in the light path and hence to the concentration of Se in the solution.

B. Statistical analysis

Data were converted 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. Frequency distribution and a percentage, means were made at first, then Chi-Square statistical test, simple correlation (r), and simple linear regression were used to test for associations between variables. A p-value <0.05 was regarded as statistically significant.

III. RESULTS

A total of ninety (90) women; 35 with RPL (unexplained consecutive pregnancy loss), 30 NAP and 25 as HC were enrolled in current study. The age of study subjects was range from 22 to 34 years with matched BMI (Table 1).

Table 1: Classification of the study groups

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

n: number.

Figure (1) depicts the VE outcomes in all study groups. The vast majority of NAP and RPL groups had the highest frequency percent of below normal level (93.3% and 88.6%, respectively) of serum VE compared to the HC group (28%)

with a significant difference (p=0.00). There was no statistically significant difference between the NAP and RPL groups. The mean titers of this biomarker were significantly (P=0.00) lower in the RPL and NAP groups (4.5 nanomoles (nmol)/ml and 4.62 nmol/ml, respectively) than in the HC group (19.67 nmol/ml). Other mean titer comparisons revealed non-significant differences.

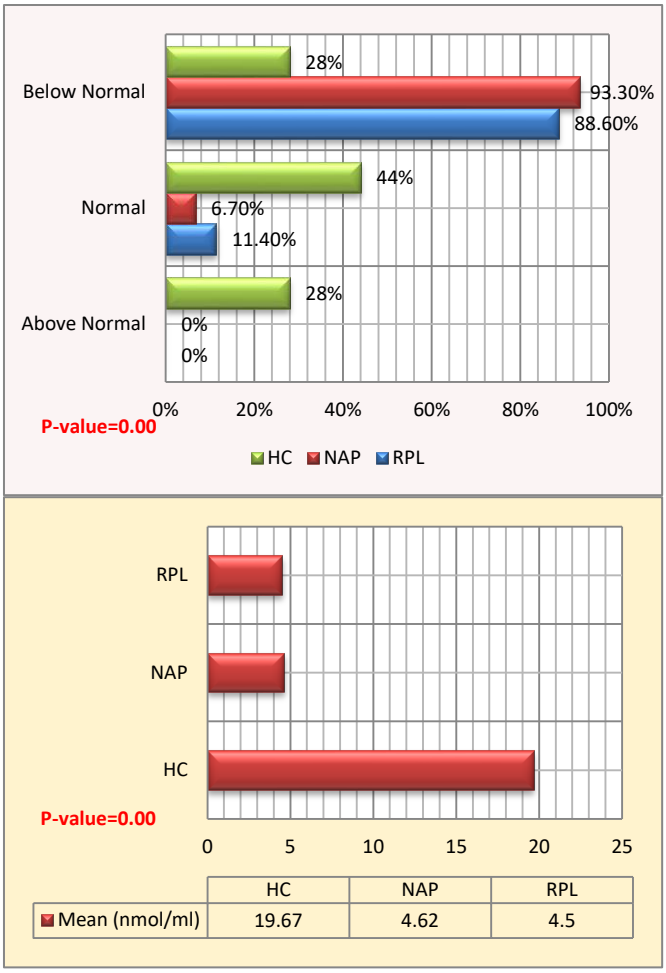

FIGURE (1): The results of frequency (%) and mean titer of vitamin E in all study groups **(nmol:** nanomoles, **ml:** milliliter, **HC**: healthy control , **NAP:** non-aborted pregnant and **RPL:** recurrent pregnancy loss).

The results of Se in all study groups were demonstrated in figure (2). The frequency percent of Se below normal level was higher in RPL group (62.9%) compared to NAP group (13.3%) and HC group (24%) with a significant differences (P=0.00). For mean titer, RPL group had exhibited the lowest mean titer (1.83 part per million (ppm)) in comparison to NAP group (2.58 ppm) and HC group (2.44 ppm) with a significant difference (P=0.00). The difference between NAP group and HC group was not significant.

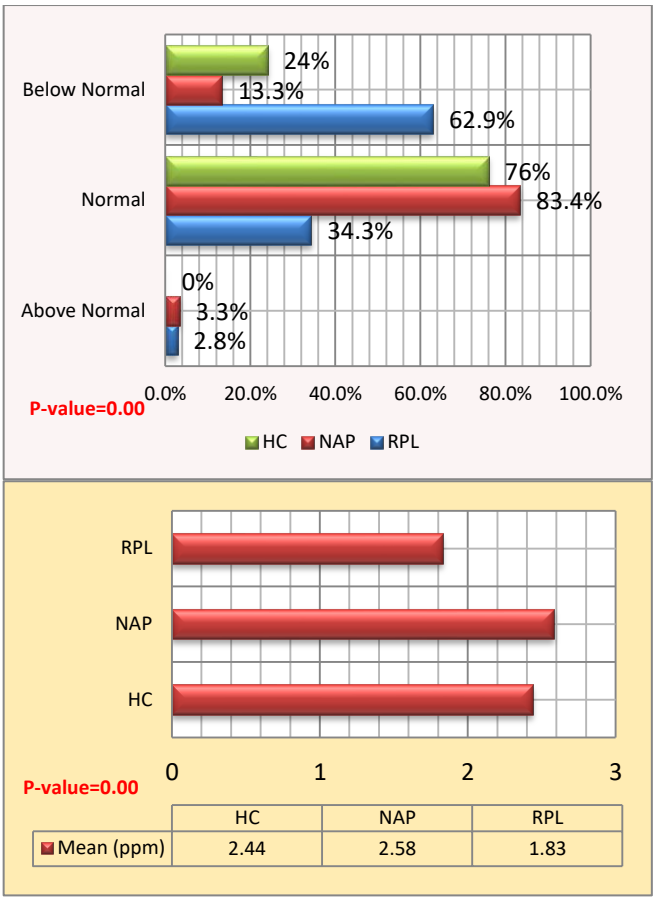

FIGURE (2): The results of frequency (%) and mean titer of selenium in all study groups **(ppm:** part per million, **HC:** healthy control**, NAP:** non-aborted pregnant and **RPL:** recurrent pregnancy loss**)**.

Table (2) showed the relationship between serum VE and serum Se in all study groups. The frequency percent of below normal serum Se level was high 21/31(67.8%) in RPL women with below normal VE level, the different was statistically significant (*p<0.05*) in comparison to women with normal VE level 1/ 4 (25%). For mean titer, the results revealed that the mean titer of Se was lower among women with below normal VE level (1.7 ppm) when compared to women with normal VE level (2.2 ppm) with a significant difference (*p<0.05*). For NAP and HC groups, women with below normal VE level had significantly (P<0.05) highest frequency percent of normal Se level 24/28 (85.7%) and 6/7 (85.7%), respectively, in comparison to the women with normal VE level 1/2(50%) and 7/11(63.6%), respectively. The same results profile was reported for mean titer within the former groups.

TABLE 2 : Correlation between vitamin E and selenium in all study groups

Parameters			Selenium (ppm)								p. valu1e
			Below N (< 2)		Normal (2-3.5)		Above N (> 3.5)		Total		
			FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	FR(%)	Mean	
Vitamin E (nmol /ml)	RPL (n=3	Below N (n=31)	21(67.8)	1.3	9(29)	2.5	1(3.2)	3.7	31(100)	1.7	<0.05
		Normal (n=4)	1(25)	1.7	3(75)	2.4	0(0)	0	4(100)	2.2	
		Total (n=35)	22(62.9)	1.5	12(34.3)	2.4	1(2.8)	3.7	35(100)	1.8	
	AP (n=3	Below N (n=28)	3(10.7)	1.7	24(85.7)	2.6	1(3.6)	3.9	28(100)	2.6	<0.05
		Normal (n=2)	1(50)	1.4	1(50)	2.7	0(0)	0	2(100)	2.0	
		Total (n=30)	4(13.3)	1.6	25(83.4)	2.6	1(3.3)	3.9	30(100)	2.5	
	HC (n=25)	Below N (n= 7)	1(14.3)	1.3	6(85.7)	8.8	0(0)	0	7(100)	9.4	<0.05
		Normal (n=11)	4(36.4)	1.6	7(63.6)	2.7	0(0)	0	11(100)	2.3	
		Above N (n=7)	1(14.3)	1.9	6(85.7)	2.5	0(0)	0	7(100)	2.3	
		Total (n=25)	6(24)	1.6	19(76)	4.6	0(0)	0	25(100)	2.4	

RPL: recurrent pregnancy loss**, NAP:** non-aborted pregnant**, HC:** healthy control**, FR:** frequency, **(%):** percent, , **n:** number, **ppm:** part per million, **nmol:** nanomoles, **ml**: milliliter and **N:** normal.

The serum VE and Se levels in the RPL group showed a significant positive connection ($p< 0.05$) according to the regression analysis in figure (3). The results demonstrated a negative correlation between the two parameters for the NAP and HC groups in the same figure, with a significant difference ($p< 0.05$).

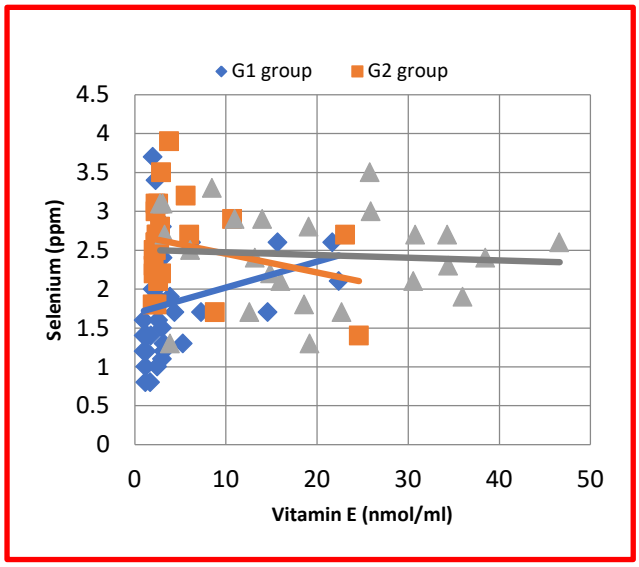

FIGURE 3: Regression analysis of vitamin E and selenium in all study groups **(G1:** recurrent pregnancy loss, **G2:** non aborted pregnant , **G3:** healthy control, **ppm:** part per million, **ml:** milliliter and **nmol:** nanomoles).

IV. DISCUSSIONS

Recurrent spontaneous miscarriage, also known as RM, chronic abortion, or RPL, is characterized as three or more recurrent miscarriages before the 20th week of pregnancy. Recurrent pregnancy loss happens in 1-5% of pregnant women. Because the etiology of RPL is uncertain, further clinical and laboratory research is necessary [14]. Previous research has found that chromosomal abnormalities, endocrine illnesses, uterine abnormalities, placental malformations, hormonal difficulties, thrombophilia, infections, nutritional disorders, autoimmune disease, and anatomy are all implicated in certain RPL instances [15, 16]. Despite multiple research studying the aforementioned criteria, the pathogenesis of RPL remains unknown. To improve live birth rates in patients with RPL, early prediction of the possible risk of RPL is essential [14]. According to the findings of the current study, women with RPL had significantly low VE levels compared HC group (Figure 1). Overall, these findings were consistent with other previous studies [7,8 and 17] had shown that a lack of VE can lead to females infertility, miscarriage, early birth, eclampsia, fetal intrauterine development restriction, and other pregnancy-related disorders. In the same figure, the findings revealed that VE levels in the NAP group wase significantly lowest than the levels in the HC group, which might be attributed to a lack of VE supplementation during pregnancy. In the present study (Figure 1), non-significant difference in serum VE level was found when comparing the NAP and RPL groups. Several research investigations have indicated that adequate VE consumption in pregnant women improves term delivery and protects and sustains the endometrial barrier from free radicals. Its deficiency has been linked to fetal death [18]. It is thought that multiparous women in our environment may have exhausted their vitamin stores due to repeated pregnancies at short intervals [19]. Another explanation for current study findings, is that the natural hemodilution of pregnancy, insufficient nutrition, and elevated oxidative stress among this cohort of pregnant women may have been responsible for the significant drop in VE seen during the pregnancy period. The antioxidant properties of VE may protect both the mother and the fetus during pregnancy by functioning as a chain-breaking antioxidant and the body's principal lipid peroxyl radical scavenger, lowering the risk of pregnancy complications [18]. Several studies have found that VE may have a positive prospective influence on female fertility and reproductive health [20].

Vitamin E is a powerful non-enzymatic antioxidant. According to the researchers, VE is the primary free radical chain terminator in the lipophilic environment due to the lipophilic feature of the tocopherol molecule. Several research investigations have indicated that

adequate VE intake during pregnancy increases term delivery and protects and maintains the endometrial barrier from free radicals. Its deficiency has been connected to fetal death [21]. In women who miscarry frequently, there was an increase in lipid peroxidation and a substantial decrease in glutathione, VE, and beta carotene levels [22].

Our findings were in line with previous findings mentioned above since VE levels were to be low in women who had miscarriages on a regular basis.

Our findings revealed that women in the RPL group had reduced Se concentrations (62.9%) in comparison with HC group (24%) (Figure 2) because Se needs to rise during pregnancy as a result of the transfer to the developing fetus [23], resulting in lower Se concentrations in maternal blood and tissues [24]. Similar to the present study findings (Figure 2), a previous study [25] showed that serum Se concentrations in the RPL were considerably lower than in the NAP and HC. In the same figure, there were non-significant variations in Se concentrations between the NAP and HC groups (13.3% and 24% and its level were elevated in both groups (Figure 2).

In harmony with our findings, a study by Kassu and colleagues' investigation found no difference in mean serum Se levels during pregnancy when compared to controls [26]. However, another study showed Pregnant women have a lower Se status than non-pregnant control women [17]. Trace element concentration variations might be caused by a variety of factors, including age, gender, body composition, soil, geographical location, food accessibility, cultural habits (geophagia), and genetics [27]. Because oxidative stress contributes to early miscarriage, lower Se levels in miscarriage women may increase free radicals and oxidative stress [28]. In previous studies, there was a rise in oxidative stress biomarkers in placental tissues of women who had miscarried [29]. Increased oxidative stress alters the blood arteries in the placenta, leading to early miscarriage [30], or oxidative stress impairs placental growth at the start of pregnancy, affecting pregnancy outcomes such as miscarriage or preeclampsia [31]. The following processes have been proposed to explain the link between miscarriage and Se; First, there is a reduction in antioxidant defense, which causes damage to cellular membranes and deoxyribonucleic acid (DNA) [32]. Second, decreased activity of the seleno-enzymes (enzymes containing Se), which reduce pro-inflammatory genes associated with negative pregnancy outcomes [30]. Studies indicate that a diet rich in Se may reduce the risk of pregnancy loss [33].

The findings of the current study showed that the Se level was significantly higher among subjects with normal VE compared to below normal subjects of RPL group (Table 2). Another intriguing finding from this study was the substantial positive relationship between VE and Se titers within the RPL group (Figure 3).

Consistent with our results, a previous study showed that the two-fold increase in dietary VE levels resulted in lower Se levels in the blood. As a result, Se levels increased in rats fed a diet devoid of VE as a compensatory reaction (as represented in RPL in the current study). On the other hand, blood Se levels were lowest as the demand for Se in preventing anti-oxidation reactions diminished due to the two-fold increase in VE consumption [34]. In harmony with former study, current study findings also showed lower levels of serum Se among subjects with normal VE levels in both NAP and HC groups (Table 2) and there was a significant negative correlation between both biomarkers within the last mentioned groups (Figure 3). The increased VE in NAP and HC may have resulted in lower Se concentrations in various organs of the body, but the total quantity of stored Se tended to grow. This study reveals the tight compensatory connection between Se and VE.

The major limitation of the present study was the small sample size. The second limitation was the actual sensitivity and specificity of the kits that were used for biomarkers measuring in patients sera. Third, the patient's selection criteria (exclusion and inclusion) had a major effect to decrease the sample size of the study, because there was strong difficulty to found patients with RPL that met it.

V. CONCLUSION

Since the frequency percent of below normal serum vitamin E and selenium levels were higher in RPL women (88.6% and 62.9% respectively)compared to the HC group (28% and 24% respectively), their combination may be a potent and inexpensive diagnostic biomarker for RPL. A significant positive association of VE and Se in RPL and a significant negative association of VE and Se in NAP and HC, which indicated a possible compensatory correlation between both biomarkers to dictate the final outcome of pregnancy. According to our conclusions, there was a possible utility to use VE and Se as supplement therapy to decrease the incidence of RPL among pregnant women.

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