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Neopterin and the Anti-Mullerian Hormone's Role in Unexplained Recurrent Pregnancy Loss

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ABSTRACT

Background: Recurrent pregnancy loss were prevalent complications in pregnancy. Immunological biomarkers as Neopterin used as a marker in recurrent pregnancyloss. Anti-Mullerian hormone is a reliable marker of the ovarian reserve. Therefore, was also considered as a marker to evaluate its role in the recurrent pregnancy losses. This study was conducted to assess whether neopterin and Anti-Mullerian hormone can be used as a marker in the condition of unexplained recurrent pregnancy loss.

Material's and Methods: A case control study was done in the Department of Obstetrics and Gynecology Salahdeen general hospital in Tikrit city from first January to thirty of August 2020. One hundred patient enrolled in the study. Study contains two groups:1- Group A as cases. 2- Group B as control group. The data collection donethrough:1-Designed closed and open-ended questionnaire, 2-Laboratory investigations of : Serum Neopterin and Anti-Mullerian hormone levels using enzyme-linked immunosorbent assay.

Results: Mean Neopterin level was significantly higher among cases (24.3±10.7)nml/lthan control group (2.9±1.1) nml/l. Mean Neopterin level increased with increasingabortion number. It was significantly higher among those with ≥5 abortions (30.02±10.04) nml/l than those with (3-4) and 2 abortions (28.7±15.29) nml/l and (20.1±0.03) nml/l. The mean Neopterin level increased with increasing age among cases and controls. Among cases was (28.7±12.03) nmol/L among those aged (31-35) year, which was higher than those aged (18-25), and those aged (26-35) years Mean Anti-Mullerian hormone level was significantly lower among cases (0.8±0.6) ng/ml than control group (5.01±2.7) ng/ml. Mean Anti-Mullerian hormone level wassignificantly higher among those with 2 abortions (1.06±0.7) ng/ml than those with(3-4) and 5 abortions (0.6±0.56) ng/ml and (0.97±0.4) ng/ml respectively. Among case the mean AMH level was lower among those aged (31-35) years (0.5±0.31) ng/ml than those aged (26-30), and (18-25) years (0.8±0.7) ng/ml, (0.97±0.7) ng/ml,respectively

Conclusions: A statistically significant high Mean Neopterin level among cases and its increased mean Neopterin level with increasing abortion number. A statistically significant low levels of mean Anti-Mullerian hormone level among cases, more reduction in its level with increasing abortion. This pioneer study in Iraq, determinedthe significant association between the unexplained recurrent pregnancy loss with the increased levels of Neopterin and its effect on Anti-Mullerian hormone that lead to recurrent pregnancy loss.

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1. INTRODUCTION

Recurrent pregnancy loss (RPL) is considered as an important reproductive health issue, since it affects 2%–5% of couples.(1) in Iraq in 2020 it was 16.5%. (2) Thedefinition of RPL has long been debated and differs among international societies. Forthe European Society for Human Reproduction and Embryology (3) and the Royal College of Obstetricians and Gynecologists, RPL refers to three consecutive pregnancy losses, including non-visualized ones. However, according to the American Society for Reproductive Medicine, it is defined as two or more clinical pregnancy losses (as documented by ultrasonography study or histopathologic examination), but not necessarily consecutive (4). Clinically recognized pregnancy loss is common,

occurring in approximately 15%–25% of pregnancies. The majority of sporadic losses before 10 weeks' gestation result from random numeric chromosome errors, specifically trisomy, monosomy, and polyploidy [(5)].

Less than 5% of women will experience two consecutive pregnancy loss, and 1% experience ≥ 3. Common established causes include uterine anomalies, antiphospholipid syndrome, hormonal and metabolic disorders, and cytogenetic abnormalities [(6)]. In concern to its pathophysiology, forkhead box D1 (FOXD1) mutations play a critical role in RPL. FOXD1 was defined as a major molecule involved in embryo implantation in mice and humans through regulation of endometrial and placental genes. FOXD1 mutations in human species have been functionally linked to RPL's origin [(6)].

Unexplained RPL is considered if occur without any anatomic, genetic, endocrine, and immune abnormalities. Unexplained RPL is associated with significant adverse psychological consequences for the couple. Besides the grief following each miscarriage, there is the anxiety and insecurity associated with each positive pregnancy test [(7)].

A variety of factors used as markers in the condition of unexplained recurrent pregnancy loss, of these markers is neopterin (NP), which emerges as a result of the cellular immunity system activation. NP production and release were observed from human monocytes and macrophages activated by interferon-gamma (IFN-c) stimulation. Therefore, NP is thought to shed light on the diagnosis of many diseases where T lymphocytes and macrophages are involved in their aetiopathology [(8)]. T- lymphocyte-2 metabolites are increased in a normal pregnancy. While, the increase in T-lymphocyte-1 metabolites may lead to poor prognostic outcomes, such as preeclampsia, spontaneous abortion, missed abortion and RPL [(8)]. On the other hand, Anti-Mullerian hormone (AMH) is a reliable marker of the ovarian reserve. Therefore, AMH was also considered in many studies to evaluate its role in the RPL cases as a marker in these patients [(9)], with subsequent studies, high levels of NP and low values of AMH in patients with RPL can be used as predictive markers for this clinical situation[(9)].

Anti-Müllerian hormone, also defined as Müllerian inhibiting substance, is essential for the involution of the Müllerian ducts (the anlagen of the internal female genitalia) in the male fetus. Male sex differentiation is completely dependent on the normal development of testes that produce ample amounts of testosterone and AMH [(10)]. These hormones, produced by Leydig cells and Sertoli cells, respectively, represent two distinct pathways in male sex differentiation. Testosterone is responsible for the differentiation of the Wolffian ducts, urogenital sinus, and external genitalia. By contrast, AMH does not have any known function in female fetalorganogenesis [(11)]. This study was conducted to assess whether neopterin (NP) and Anti-Mullerian hormone (AMH) can be used as a marker in the condition of unexplained recurrent pregnancy loss (RPL).To evaluate the relation between neopterin (NP) and unexplained recurrentpregnancy loss (RPL). To determine the relation between anti-mullerian hormone (AMH) and unexplainedrecurrent pregnancy loss (RPL). To assess the relation between hematological markers and unexplained recurrentpregnancy loss (RPL).

2. MATERIAL'S AND METHODS

2.1. Study Design

Case control study, The study was done in the Department of Obstetrics and Gynecology Salahdeen general hospital in Tikrit city. From 1st Jan. 2020 - to the end of August. 2020

2.2. Sampling and Sample Size

One hundred patients enrolled in the study which contains 2 groups: Group A as cases consist of 50 women with recurrent abortion, at least2 consecutive spontaneous miscarriage (≤ 13 week). Group B as control group: fifty women with a minimum one full-termbaby without history of pregnancy complications.

2.3. Inclusion Criteria

The inclusion criteria included the following: Group A: cases inclusion criteria: history of spontaneous abortion of 2or more miscarriages at ≤ 13 week of gestation. Group B: control group: women with at least one full term babywithout complicated pregnancy

2.4. Exclusion criteria for cases group

The exclusion criteria include: 1)Patients with obvious cause of recurrent miscarriage e.g hypothyroidism, DM, uterine congenital anomalies, history of pulmonary thromboembolism or deep vein Thrombosis, coagulation defects, thrombophilia. 2) Autoimmune diseases 3) Chromosomal abnormalities. 4) Conditions that cause elevated NP either acute or chronic inflammatory illness 5) Medications history 6) Past history of surgical interventions which affect ovarian reserve(e g. cystectomy). 7)PCOs

2.5. Data Collection

The data collection done through:

1- Designed closed and open-ended questionnaire, done by the researcher(Appendix I), by using direct interviewing, contain information about features of patient demography such as , age, parity, gravidity, abortions, past surgical and medical history, congenital anomalies of the uterus e.g bicornate, arcuate and fibroid, family histories and consanguineous marriage, smoking, thrombophilia , PCOs history, and pregnancy complication history.

2- Laboratory investigations of: S. Neopterin & AMH levels using enzyme-linked immunosorbent assay (ELISA). All venipuncture precaution was taken. The blood chemical; integrity was preserved from the time of collection to the analysis. All patients were investigated for the levels of NP, and AMH therefore; 4ml of blood were collected. For serum separation the samples were centrifuged for 10 min (10,000 rpm/min.). Obtained serums were transferred to 2ml

Eppendorf tubes (Eppendorf, Hamburg, Germany) and then preserved in 80 C freezer until the analyse time. Samples were protected from light during these procedures.

2.6. Statistical Analysis

Statistical package of social science (SPSS) Software version 23.0 was used for data analysis. Percentages and mean was used to present the datain tables. Comparison of study groups was carried out using chi-square test for categorical data, and Student's t-test for continuous data. P-value of < 0.05 was considered statistically significant.

3. RESULTS

3.1. General characteristics of study groups

The age distribution shows that most of the cases were age 31-35 years, whilemost of the controls were aged 26-30 years 20(40%), this relation was statistically not significant as shown in table 3.1.

BMI of the cases were 12(24%) of them 25-24.9 kg/m^2, 16 (32%) had BMI 30-35 kg/m^2, and 8(16%) had >35 kg/m^2. This was higher than of the control group,20 (40%), 12(24%), and 4(8%) respectively, this relation was statistically not significant. Family history was positive among 20(40%) of the cases as compared with 8(16%) of the control group, this relation was statistically significant as shownin table 3.1.

Infertility history was higher among cases primary infertility 14(28%), secondary infertility 12(24%), than the control group 6(12%), and 2(4%) respectively, this relation was statistically significant. Consanguinity was positive among 31(62%) of the cases which is higher than controls 17(34%), this relation wasstatistically significant as shown in table 3.1.

Smoking found among 8(16%) of the cases and 4(8%) of the controls this relation was statistically not significant as shown in table3.1.

Table 3.1. General characteristics of study groups

		cases		Control		Total		P value
		Freq.	%	Freq.	%	Freq.	%	
Age in years of enrolled women	18-25 Years	12	24%	14	28%	26	26%	0.46
	26-30 Years	16	32%	20	40%	36	36%	
	31-35 Years	22	44%	16	32%	38	38%	
BMI	<18 kg/m^2	4	8%	2	4%	6	6%	0.3 LikelihoodRatio
	18-24.9 kg/m^2	10	20%	12	24%	22	22%	
	25-24.9 kg/m^2	12	24%	20	40%	32	32%	
	30-35 kg/m^2	16	32%	12	24%	28	28%	
	>35 kg/m^2	8	16%	4	8%	12	12%	
Family History RPL	Negative	30	60%	42	84%	72	72%	0.008*
	Positive	20	40%	8	16%	28	28%	
Infertility Negative	Negative	24	48%	42	84%	66	66%	0.001*
nfertilitypositive	primary Infertility	14	28%	6	12%	20	20%	
	secondary Infertility	12	24%	2	4%	14	14%	
Consanguinity	Negative	19	38%	33	44%	22	22%	0.005*
	Positive	31	62%	17	34%	48	48%	
Smoking	No	42	84%	46	92%	88	88%	0.21
	Yes	8	16%	4	8%	12	12%	

*****significant, RPL: recurrent pregnancy loss,

Most of the cases were nulliparous 32(64%), followed by those delivered onebaby 16 (32%). while among controls most of the women had ≥3 child 34 (68%), this relation was statistically significant as shown in table 3.2.

3.2. The distribution of study groups according to the number of parity.

Parity	Cases(50)		Control(50)	
	Freq.	%	Freq.	%
0	32	64	0	0
1	16	32	4	8
2	2	4	12	24
≥3	0	0	34	68
Total	50	100	50	100

x^2=80.343, df=3, p value=0.0001 significant

The frequency of abortions among cases was 2 among 16 (32%), followedby 3 abortions among 14 (28%), 4 abortions among 14(28%), and ≥ 5 abortions among 6 (12%), as shown in figure 3.1.

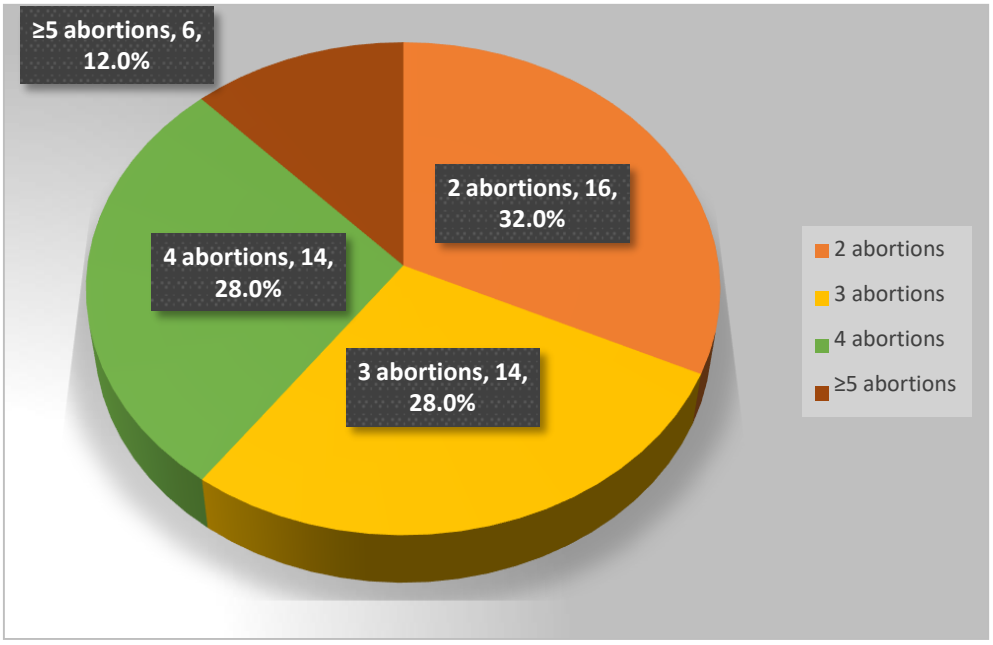

Figure 3.1. The number of abortions among cases group

3.2. The neopterin and anti Mullerian hormone relation withrecurrent abortion

Mean neoptrin nmol/L level was higher among cases (24.3±10.7) nmol/Lthan control group (2.9±1.1) nmol/L, this relation was statistically significant asshown in table 3.3**.**

Table 3.3. The neopterin level among the Study Groups

Study Group	N	Minimum	Maximum	Mean± SD	student test t P value
Cases	50	10.2	49.11	24.3±10.7	0.001*
Control	50	1.12	5.39	2.9±1.1	

* highly significant.

The mean neopterin level increased with increasing abortion number. It was higher among those with ≥5 abortions (30.02±10.04) nmol/L than those with (3-4)and two abortions (28.7±15.29) nmol/L and (20.1±0.03) respectively, this relationwas statistically significant, as shown in table3.4.

Table 3.4. The relation of neopterin level and number of abortions amongcase group.

No. of abortions	N	Minimum	Maximum	Mean± SD	P value ANOVA
2	16	10.2	41.9	20.1±0.03	0.004*
3-4	28	18.15	48.45	28.7±15.29	
≥5	6	21.02	49.11	30.02±10.04	
Total	50	10.2	49.11	24.25	

*significant

The mean neopterin level increased with increasing age among cases and controls. Among cases was (28.7±12.03) nmol/L among those aged (31-35) year,which was higher than those aged (18-25), and those aged (26-35) years, this relation was statistically significant as shown in table 3.5.

Table 3.5. The relation of neopterin level and age among both groups.

Age	cases Mean± SD	controls Mean± SD
18-25 years	18.2±5.4	2.5±0.8
26-30 years	22.7±9.3	2.8±1.5
31-35 years	28.7±12.03	3.25±0.8
Total	24.3±10.7	2.85±1.13
P value ANOVA	0.016	0.19

Mean AMH level was lower among cases (0.8±0.6) ng/ml than control group (5.01±2.7) ng/ml, this relation was statistically significant as shown in table3.6**.**

Table 3.6. The AMH of the Study Groups

Study group	N	Minimum	Maximum	Mean± SD
Cases	50	0.06	2.3	0.8±0.6
Control	50	1.8	11.8	5.01±2.7

t-student test t =-10.88, df=98, P value =0.001 highly significant.

The mean AMH level ng/ml was higher among those with two abortions (1.06±0.7) ng/ml than those with (3-4) and five abortions (0.6±0.56) ng/ml and (0.97±0.4) ng/ml respectively, this relation was statistically significant, as shownin table 3.7

Table 3.7. The relation of AMH level ng/ml and number of abortions amongcase group.

No. of abortions	N	Minimum	Maximum	Mean± SD	P value ANOVA
2	16	0.06	2	1.06±0.7	0.04*
3-4	28	0.12	2.3	0.6±0.56	
≥5	6	0.4	1.26	0.97±0.4	
Total	50	0.06	2.3	0.78±0.6	

*significant

Among case the mean AMH level was lower among those aged (31-35) years (0.5±0.31) ng/ml than those aged (26-30), and (18-25) years (0.8±0.7) ng/ml, (0.97±0.7) ng/ml, respectively, this relation was statistically significant, as shown in table 3.8.

Table 3.8. The relation of AMH level and age among both groups.

		cases	controls
		Mean± SD	Mean± SD
AMH	18-25 years	0.97±0.7	6.5±3.1
	26-30 years	0.8±0.7	4.07±1.7
	31-35 years	0.5±0.31	3.98±1.9
	Total	0.78±0.6	5.0±2.7
	P value ANOVA	0.07*	0.004*

*significant

3.3. The Hematological Characteristics of the Study Groups

The mean WBC was higher among cases group (11.05±4.8) (/mm3 $\times 10^3$) than control group (9.7±3.8) (/mm3 $\times 10^3$), this difference was not statistically significant. Mean lymphocyte % among cases was (24.6±12.6), compared to the control group (25.9±16.7) this relation was not statistically significant. Mean lymphocyte count among cases was 2.5±1.6 x 10^3 lymphocytes/mcL, compared to the control group 5.2±6.1 x10^3 lymphocytes/mcL, this relation was statistically significant. Mean granulocyte % among cases was (64.8±15.2), compared to the control group (62.8±16.1), this relation was not statistically significant. Mean granulocyte count among cases was 7.1±3.9x10^3 lymphocytes/mcL, compared to the control group 6.6±3.4x 10^3 lymphocytes/mcL, this relation was not statistically significant. Meanneutrophil /Lymphocyte Ratio among cases was (3.7±1.1), compared to the controlgroup (3.1±0.4), this relation was statistically significant. Mean PLT among cases was (255.5±75.2), compared to the control group (216.2±62.1), this relation was statistically significant. As shown in table 3.9. Mean platelet/lymphocyte Ratio among cases was (127.3±51.3), compared to the control group (94.2±44.1), this relation was statistically significant. As shown in table 3.9.

3.9. The Hematological Characteristics of the Study Groups

Hematologicalcharacteristics	**Cases**	**Control**	**P value (t)**
	Mean ±SD	**Mean ±SD**	
WBC (/mm3 ×10^3)	**11.05±4.8**	**9.7±3.8**	**0.1(1.5)**
Lymphocyte %	**24.6±12.6**	**25.9±16.7**	**0.6(0.4)**
Lymphocyte count (10^3 lymphocytes/mcL)	**2.5±1.6**	**5.2±6.1**	**0.004(2.96)***
Granulocyte %	**64.8±15.2**	**62.8±16.1**	**0.5(-0.63)**
Granulocyte Count(10^3 Granulocyte /mcL)	**7.1±3.9**	**6.6±3.4**	**0.4(0.67)**
Neutrophil /LymphocyteRatio	**3.7±1.1**	**3.1±0.4**	**0.0005(3.6)***
PLT	**255.5±75.2**	**216.2±62.1**	**0.005 (2.8)***
Platelet/Lymphocyte Ratio	**127.3±51.3**	**94.2±44.1**	**0.0008(3.45)***

*Significant

4. DISCUSSION

4.1. General characteristics of study groups

Most of the cases were non-significantly had older age 31-35 years (44%), than controls were aged 26-30 years (40%), this agree with Rifat AG and Salman YJ [12] (2017) in Kirkuk in Iraq found that most of the patient with recurrent pregnancy loss aged >35 years 59% and those < 35 years was 41% , while of the women withoutrecurrent loss 55%, 45%respectively, further more found that this difference was not significant. [13] Oğlak SC *et al* (2020) who found a non-significant difference

between the two groups in terms of age 23 and 26 respectively, and BMI (23.12 ± 3.66) kg/m^2, and (23.78 ± 3.82) kg/m^2 respectively [64]

Also Siristatidis C *et al* [14] (2019) whom found a non-significant difference regarding age and BMI. The mean BMI among those with abortion and those with live birth was (22.2 ± 3.0) kg/m^2, and, (23.5 ± 3.5) kg/m^2 respectively, common age group among those with abortion was 30-34 years (53.8%) versus (46.8%) among those without recurrent abortion.

Chaithra PT, *et al* [15] (2015) whom considered increased mother age as a major inducing reason for RPL. Mother age at delivery is a risk factor of Down syndrome and has a major effect in recurrent pregnancy loss. [15]

There were significant difference regarding parity most of the cases were nulliparous 32 (64%), while most of the controls had ≥3 child 34 (68%). This goes with Sencan H *et al* whom found statistically significant difference in the median of parity 1.0 (0–3) and 2.0 (1–4) of those with pregnancy loss and control group, respectively [16] while disagree with Oğlak SC *et al* [13] (2020) who found a non significant difference in relation to parity. The increased parity among control group is an expected finding as those without miscarriage and our community preferred having more children.

4.2. The Neoptrin relation with recurrent pregnancy loss

Mean Neoptrin level was higher significantly among cases (24.3±10.7) than control group (2.9±1.1). This concometent with previous studies that found asignificant higher level of Neoptrin among women with recurrent pregnancy loss, as Sencan H *et al* [16] (2019) who found significantly higher level of Neopterin in thepatient group (1.69 ± 0.486 vs. 1.38 ± 0.431 ng/ml). Also agrees with Ünüvar S, andTanrıverdi Z[17] (2017) found that mean serum neopterin among those with recurrent pregnancy loss were 16.47±0.095 nmol/L higher than among control group(6.14±0.041) nmol/L. Another study done by Kuon R J *et al* [18] (2015) reported higher level of Neoptrin among those with recurrent pregnancy loss (6.82 ±0.46) than those without loss (5.38±0.25).

Neoptrin is a molecule that demonstrates increased inflammatory reaction in infectious and autoimmune disease. This increment may be related to presence of some sort of autoimmune disease in those with recurrent pregnancy loss, or may berelated to subclinical endometritis, the exclusion of those patient supported the relation of neopterin with recurrent pregnancy loss. [18]

Siwetz M *et al.* [19] 2016 reported that an increase in humoral immune responseand a depression of cell-mediated immune response are essential for healthy pregnancy. Decidual T-lymphocyte-2 (Th-2) products, is well known to rise in uncomplicated pregnancies. In cases of preeclampsia, missed abortion, spontaneouspregnancy loss, and recurrent pregnancy loss, The T-lymphocyte-1 increases (Tlymphocyte 2), this fact also support findings of this study.

The effect of immune mechanisms was also associated with the gestational period during which the abortion occurred. During preimplantation and until the endof implantation, cell-mediated immunity is said to be responsible for early abortion. Immunocompetent decidual cells or cytokines had been reported to be responsible for these immunological mechanisms. The production of IFN-γ activates decidual macrophages, causing injury by stimulating the production of nitric oxide and TNF- α, which cause apoptosis and inhibit the secretion of granulocyte macrophage colony stimulating factors from the uterine epithelium.Th1 cytokines, which are secreted as a result of IFN-γ activity, result in the termination of pregnancy through embryo and trophoblast toxicity.[17]

The mean neoptrin level increased with increasing age among cases and controls. Among cases was (28.7±12.03) among those aged (31-35) year, which washigher than those aged (18-25) (18.2±5.4), and those aged (26-35) years (22.7±9.3).This goes with Sencan H *et al* [16] (2019) and No RG [20] (2011) who found a significant increase in age progresses and increase in NP levels. But disagree with Ünüvar S *et al* [17] (2019) found a non- significant correlation with age. There wasa negative correlation between serum neopterin level and age in both the control (R=0.0774, p=0.6236) and recurrent abortion groups (R=0.1415, p=0.2089). This may be explained by what found by No RG reported that the risk of abortion increases after each consecutive pregnancy loss and the prognosis worsens with increased maternal age. In advanced age pregnancies, the risk of abortion increaseswith the decrease in the number and quality of oocyte present. The risk is higher particularly in women over the age of 35 year.[20]

4.3. The anti Mullerian hormone relation with recurrent miscarrage

Mean AMH level was significantly lower among cases (0.8±0.6) than controlgroup (5.01±2.7). This goes with Pils S *et al* [21] (2016) whom reported that Anti- Mullerian hormone was significantly lower in women with idiopathic recurrent miscarriage (median 1.2 ng/ml) than in women with explained recurrent miscarriage(median 2.0 ng/ml). Another study in 2019 [16] reported that mean level of AMH (1.38 ± 0.683 ng/ml) in the patient's group was significantly lower than the control group (1.84 ± 0.718 ng/ml).

The mean AMH level was significantly higher among those with two abortions (1.06±0.7) than those with (3-4) and five abortions (0.6±0.56) and (0.97±0.4) respectively, this goes with Sencan H *et al*[16] (2019) who found a significantly decrease in AMH with increasing age of abortions, patient with > two abortions (1.78 ± .612), and those >3 abortions was (1.13 ± .606). Pils S *et al* [21] (2016) whom found that further miscarriages occurred in whom a higher number ofprevious miscarriages was predictive (OR 3.568, 95% CI 1.457–8.738).

This may explained by what Choi TY *et al* [22] (2014) who found, that a highrate of about 5–75% of miscarriages is associated with embryonic chromosomal abnormalities, also

caused by decreased oocyte quality. Various studies have demonstrated increased rates of chromosomal abnormalities in embryos derived from couples with RPL. Among case group the mean AMH level was significantly lower among those with older aged (31-35) years (0.5±0.31) than those aged (26- 30), (18-25) years (0.8±0.7), (0.97±0.7). This goes with Sencan H *et al* [16] 2019 who found non significantly decrease in AMH with increasing age, it was (1.52 ± .702) among those aged 18-25 years, while was (1.30 ± .752) among those aged 31-35 years.

This results disagree with Revelli, A *et al* [23] (2016) who confirmed that the affection of pregnancy success by age and not the small difference in AMH level using logestic regression. Which was significantly affected by age, but not by smalldifferences in AMH level. A large prospective study done by Kedem A *et al* (2013)reported that AMH in its lower range (0.2–1 ng/ml) was a reliable quantitative marker of the ovarian follicular pool, but performed poorly as a predictor ofpregnancy. Of note, no pregnancy was obtained in women above 42 years, suggesting that age, rather than AMH, is the main factor to be Considered when dealing with patients with AMH in such a low range.[24]

4.4. The Hematological Characteristics of The Study Groups

There was a non- significant difference between cases and controls regardingWBC it was 11.05±4.8 among cases and 9.7±3.8 among control group. This goes with Siristatidis C *et al* [14] (2019) who found the same finding (7.71 ± 2.16), and (7.62 ± 2.54) respectively among cases and controls.

In this study the mean Lymphocyte count among cases was 2.5±1.6 x 10^3 lymphocytes/mcL, which was significantly lower than control group 5.2±6.1 x 10^3 lymphocytes/mcL, this goes with Oğlak S C *et al* [13] (2020) found significant lower lymphocyte count among females with RPL 1.6 x 10^3 lymphocytes/mcL, versus 2.3 x 10^3 lymphocytes/mcL for normal pregnancy. But this disagree with Siristatidis C *et al*[65] (2019) found a non-significant difference regarding lymphocyte count amongthose with live birth and those with abortion (2.19 ± 0.43), and (2.75 ± 2.55) respectively.

Mean Neutrophil /Lymphocyte Ratio was significantly higher among cases (3.7±1.1), than control group (3.1±0.4). This goes with Oğlak S C *et al*[13] (2020) found significant higher NLR among cases than controls 3.5, and 1.9 respectively, and with Charalampos Siristatidis *et al*[14] (2019) 13.82 ± 4.81 ,12.62 ± 1.03 respectively. This may be explained by the fact that neutrophil /lymphocyte Ratio is an inflammatory response indicator. It was reported that high endometrial cytokines, leukocytes, and chemokines were associated with inflow of dendritic cells, macrophages, and pro inflammatory cytokines. This process induce a good effect bymode of trophoblast migration or by attract macrophages and dendritic cells to the site of implantation, those will induce more chemokines and cytokines production, in its turn recruiting the implantation site with dendritic cells and macrophages[26-28] . Mean PLT among cases was significantly higher among cases (255.5±75.2), than control group (216.2±62.1), this goes with Al-Aghbary AA *et al* [29] (2018) whofound that Platelets count (253.80±64.60) among those with recurrent loss, while among normal pregnancy it was (213.60±71.90). While Oğlak SC and Aydın MF [13](2020) who found non- significant increase in median platelet count among those with RPL and normal pregnancy (264.1), and (257.8) respectively.

Mean platelet/lymphocyte Ratio among cases was (127.3±51.3), compared to the control group (94.2±44.1), Oğlak SC [64] (2020) found significant increase in Platelet/Lymphocyte Ratio among those with RPL and normal pregnancy (150.7), (84.1) respectively. These differences are similar to that recently reported in India by Meena *et al* [30] (2017), in Turkey by Avcıoğlu *et al* [31](2014), and Dundar *et al* [32] (2015). There were many researches related these relation to the platelet indices and the increased risk of thrombosis. It was considered as multifactorial and it related tohemodilution, increased platelet consumption and increased platelet aggregation driven by the increased level of thromboxane A2[33].

CONCLUSIONS

1. High levels of NP and low values of AMH in patients with RPL can be used as predictive markers for this clinical situation.
2. If the causes of high levels of NP and low levels of AMH can be better illuminated, new treatments towards these causes can be developed to helpsuch patients become childbearing.
3. The number of abortion increased with increased neoptrin level anddecreased AMH.

RECOMMENDATIONS

1. There is a need for further studies including molecular and geneticexaminations to clarify the etiopathogenesis.
2. Further studies with large number of patients and multi-centered are wanted.
3. Further studies wanted to find out an international assay standard for AMHmeasurements.
4. Neoptrin and anti mulerian hormone should be included in the protocol ofrecurrent pregnancy loss management.

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