Relation between the Recurrent Miscarriages in Women with Polycystic Ovary Syndrome and the Level of INHIBIN B Hormone

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Abstract

Recurrent miscarriage, which is defined as the loss of two or more pregnancies in a row, affects 1% of couples trying to conceive. It has believed that 1–2% of first and second-trimester pregnancies end in miscarriage before the 24-week stage. PCOS (polycystic ovarian syndrome) is a prevalent endocrine condition that affects women of reproductive age. Women with PCOS are at risk for fertility issues (menstrual cycle disorders, failure to ovulate, late menopause, endometrial cancer, and infertility). Objective:- The purpose of this study were to evaluate the levels of INHIBIN B hormone in the serum of women who suffer from recurrent miscarriage with PCOS and to compare it with women of the control group, in addition to verifying its relation with obesity and maternal age. Subjects and method: - A case and control study includes 90 women in reproductive age (15-45), 50 of them had polycystic ovarian syndrome diagnosed, and 40 female were control group healthy. Samples were taken from October 2021 to March 2022, and INHIBIN B levels had analyzed by absorbance ELISA microplate reader and ELISA microplate washer from BioTek company in USA. INHIBIN B concentration was measured with a body mass index (BMI) calculated from weight divided by height in square metres (kg/m²). Result: - The levels of INHIBIN B in patients women that significantly decreased when compared with the control group (P=0.01). In addition, the level of INHIBIN B concentration in control group were the highest in Body Mass Index than patients with PCOS. Conclusion: - INHIBIN B level is decrease in PCOS patients. BMI proportion in patients was higher percentage in obese women. A high level of INHIBIN B was found in the first month of pregnancy. In addition, this hormone is associated with an increase in the number of recurrent miscarriages, where the hormone concentration on the date of the last miscarriage from one to four months is higher than the longest period of time.

Keyword: Recurrent miscarriages, polycystic ovary syndrome, INHIBIN B hormone

1. Introduction

The expression miscarriage refers to the loss of a pregnancy before the embryo reaches viability. As a result, the term encompasses all miscarriages from conception until 24 weeks of pregnancy. Recurrent miscarriage, which is defined as the loss of two or more pregnancies in a row, affects 1% of couples trying to conceive. It has believed that 1–2% of first and second-trimester pregnancies end in miscarriage before the 24-week stage [1]. Infertility and miscarriage are two types of reproductive failure they have almost the same reasons, according to experts. Polycystic ovary syndrome disease, uterine septum, and uterine fibroid are just a few of the diseases, which are related to both infertility and miscarriage. Patients who have had a recurrent miscarriage have a higher likelihood of infertility [2]. PCOS (polycystic ovarian syndrome) is a prevalent endocrine condition that affects women of reproductive age [3]. The prevalence of PCOS in women of reproductive age ranges from 5% to 18%, according to a systematic review and meta-analysis [4]. High levels of androgens, ovarian dysfunction, and polycystic ovaries are all symptoms of PCOS, albeit there are substantial differences between people [5]. This multi-factorial condition first manifests throughout puberty. Women with PCOS are at risk for fertility issues (menstrual cycle disorders, failure to ovulate, late menopause, endometrial cancer, and infertility), metabolic issues (insulin resistance, diabetes type 2, hypertension, and cardiovascular diseases), physical issues (central obesity, acne, hair loss, and baldness), and psychological issues (depression, stress and anxiety) [6]. Menstrual dysfunction and clinical or laboratory hyper androgen level are the two main components for diagnosing this condition, and these elements are employed in clinical diagnosis [7]. Most PCOS individuals only have one or two clinical symptoms. Monthly disorders are the most prevalent clinical finding, which generally begin at or shortly after menarche and can manifest as hypo menorrhea, amenorrhea, or poly menorrhea, until the menstrual cycle is regular [8, 9]. The research suggests that this syndrome is a condition that manifests in teens because of inherited ovarian dysfunction to androgen secretion that is excessive, and there is evidence that PCOS has a hereditary foundation. When the embryo existence and physiologically

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ovary stimulation by the hypothalamus-pituitary axis during the birth period and at the start of adolescence [10]. Many women with PCOS have an elevated LH/FSH ratio, according to hormonal measurements [11]. The polycystic ovary is bigger, has more follicles, and has a particularly thick core tissue where testosterone produced, compared to the normal ovary. The normal ovary has five follicles on average and is around the size of a walnut. The polycystic ovary has 10 or more follicles, which are usually tiny follicles measuring two to ten mm in diameter. Polycystic ovarian cysts are normally the size of a hen's egg, but they can also reach the size of an orange. The increased size of the polycystic ovary is primarily due to an increase in tissue, rather than, as one might assume, because of increased ovarian size of the cysts or additional follicles, the follicles are usually too tiny to make a significant contribution to the size of the ovary [12, 13]. INHIBIN B is a gonadal dimer polypeptide hormone that regulates the synthesis and secretion of follicle stimulating hormone (FSH) in a negative feedback loop. Its bioactivity is thus dependent on the creation of a dimer structure. A hormone regulates the hypothalamic-pituitary-gonadal axis. Throughout pubertal development and childhood [14]. INHBB levels are a better indicator of infertility result of recurrent miscarriages than FSH and LH [15]. INHBB serves a physiological role in reproductive endocrinology applications in both men and females, according to decades of research. In a woman, it is regarded as a more advanced marker. As it is closely related to the number of ovarian follicles, it is sensitive for ovarian follicle number [16]. A higher level of serum INHBB in reproductive-age women is one of the key determinants in maintaining a low serum FSH level. However, as they become older, the quality and quantity of their ovarian follicles decline, the level of serum INHBB declines gradually, and the inhibitory impact on FSH is diminished, which is one of the major causes for their serum FSH levels steadily rising [17, 18]. When women experience weight gain, anorexia nervosa, and an increase in adipose tissue their INHBB levels rise. Researchers gradually understood the role of INHBB in female fertility after years of research and discovered that a decrease in INHBB was the earliest sign of follicle number loss as women aged [19].

2. Subject and Material

This study was carried out by chemical department, College of education for pure science, University of Karbala and the cases individuals were obtained from Gynecological and Obstetric Teaching Hospital from the period October 2021 to March 2022 study included 50 patients women with PCOS and suffer from recurrent miscarriage, and another 40 apparently healthy women (controls) with age range (15 - 45) years. Women with PCOS were diagnosed by using the Rotterdam criteria from 2003 that involved polycystic ovaries (ovulation), ultrasound, biochemical parameter and clinical signs of hyper androgen level. Blood samples are taken from non-pregnant women from Gynecological and Obstetric Teaching Hospital and outpatient clinics for the purpose of medical tests for fertility hormone (INHIBIN B). Five ml of blood was taken using a 5 ml medical syringe and the blood was placed in gelatine tubes free of anti-clotting material, as it contains a gelatinous substance that helps to increase the separation of serum formed after the centrifugation process. The samples were left for 15 minutes at room temperature, after which they were inside a centrifuged at a speed of 2500 (round / minute) for 10 minutes to obtain the serum that was stored at (-20)° C, unless it was used immediately.

3. Results

The Statistical Package for the Statistical Analysis System (SAS) 2012 program was used to examine the data of study. It was designed to make comparisons and use significant differences. It was considered to be significant when p≤0.05 presented as mean ±SD (standard deviation). Independent T-test statistics were applied for parameters to compare between patients and control groups.

Table (1): Comparison of INHIBIN B concentration between patients women and control group

P Value	mean ±SD	subject	parameter
N. S	12.38 ± 64.32 12.64 ± 67.16	Control patients	Age (Years)
N. S	4.85 ± 25.70 4.60 ± 25.94	Control patients	BMI ($\mathbf{kg/m^2}$)
0.01	10.00 ±133.64 10.93 ± 115.56	Control patients	INHIBIN B (**pg/ml**)

SD: standard deviation; BMI: Body Mass Index; NS: t-test p- value ≥ 0.05; No. of patients group=50; No. of control group=40

According to the presented data show mean of INHIBIN B levels (115.56 ± 10.93pg/ml) in patients for age (15-45) years and (133.64 ± 10.00pg/ml) in control group, that significantly decreased in patients' group (P=0.01) when compared with control group. The measurement of age level significantly increased of patient's women, the body mass index level almost equal in patient's women when compared with control group.

Table (2): Comparison of INHIBIN B concentration between patients and control for age (15-29) years

P Value	Mean± SD	subject	parameter
N. S	12.61 ± 60.95 10.76 ± 61.95	Control (15-29) Patients (15-29)	Age (Years)
N. S	4.80 ± 24.51 3.99 ± 23.65	Control (15-29) Patients (15-29)	BMI ($\mathbf{kg/m^2}$)
0.01	6.69 ±135.87 9.93 ± 117.00	Control (15-29) Patients (15-29)	INHIBIN B (**pg/ml**)

SD: standard deviation; BMI: Body Mass Index; NS: t-test p- value ≥ 0.05; No. of patients group=50 ; No. of control group=40

According to the presented data show mean of INHIBIN B levels (117.00± 9.93pg/ml) in patients for

age (15-29) years and (135.87 ± 6.69pg/ml) in control group, that significantly decreased in patients group (P=0.01) when compared with control group. The measurement of age level insignificantly increased of patients women, the body mass index level insignificantly decreased in patients women when compared with control group.

Table (3): Comparison of INHIBIN B concentration between patients and control for age (30-45) years			
P Value	Mean± SD	subject	parameter
0.03	11.06 ± 68.44 9.83 ± 76.05	Control (30-45) Patients (30-45)	Age (Years)
N.S	4.63 ± 27.15 3.13 ± 29.36	Control (30-45) Patients (30-45)	BMI (kg/m^2)
0.01	12.65 ±130.92 12.22 ± 113.39	Control (30-45) Patients (30-45)	INHIBIN B (**pg/ml**)
SD: standard deviation; BMI: Body Mass Index; NS: t-test p- value ≥ 0.05; No. of patients group=50; No. of control group=40			

According to the presented data show mean of INHIBIN B levels (113.39 ± 12.22pg/ml) in patients for age (30-45) years and (130.92 ± 12.65pg/ml) in control group, that significantly decreased in patients group (P=0.01)when compared with control group. The measurement of age level significantly increased of patients women (P= 0.03), the body mass index level significantly increased in patients women when compared with control group.

Body Mass Index

We used Pie charts to describe the proportion of patients for each body mass index as normal weight 18.5-24.9 kg/m² has lowest percentage obtain 27% of patient groups, over weight (pre obesity) 25-29.9 kg/m² was 34% of patients and obesity over 30 kg/m² obtain higher percentage 39% of patients groups that's mean most of patient PCOS have high BMI, as shown in figure (1).

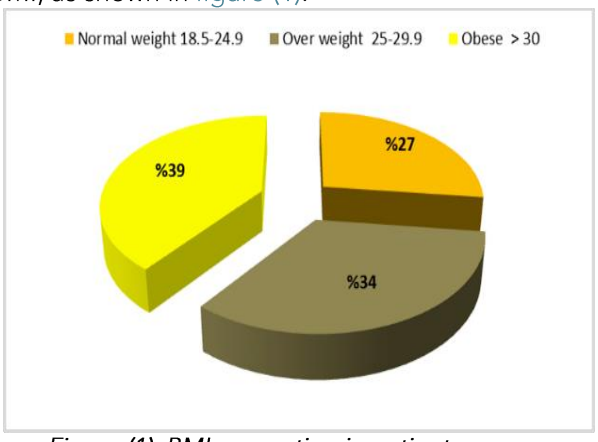

Figure (1): BMI proportion in patients groups.

Effect of BMI on INHIBIN B concentration in patients and control group

The study found that the level of INHIBIN B concentration in control group were the highest in Body Mass Index (BMI) , whether the women were of normal weight, over weight and obese when compared with patients women, as shown in figure (2).

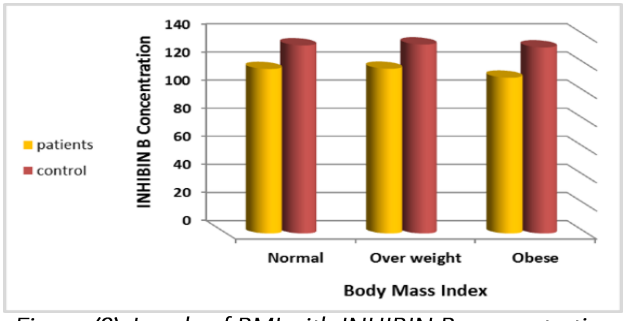

Figure (2): Levels of BMI with INHIBIN B concentration in patients and control group.

Effect of the duration of pregnancy on INHIBIN B level in patients group

we used the relation between duration of pregnancy and concentration of INHIBIN B of patients women, and its effect on these hormone in one month, two month and three month or more, the measurement showed that the level of these hormone was higher in one month when compared with two and three month, as shown in figure (3).

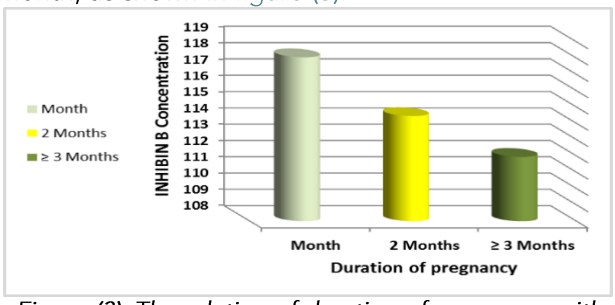

Figure (3): The relation of duration of pregnancy with INHIBIN B concentration.

Influence of number of miscarriages on INHIBIN B concentration in patients women

Patients group were categorized according to the number of miscarriages into women with two, three or more miscarriages and its relation with the level of INHIBIN B, it was found that the level of INHIBIN B in women with three miscarriages or more is higher than in women with two recurrent miscarriages, as shown in figure (4).

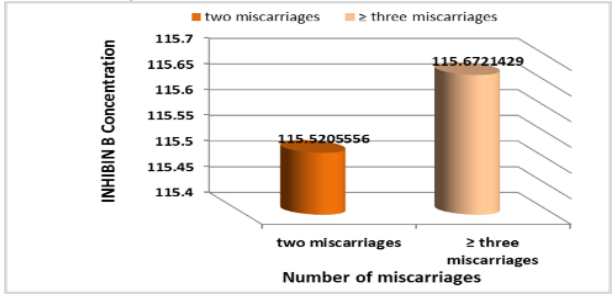

Figure (4): The relation of number of miscarriages with INHIBIN B concentration.

Influence of last miscarriages date on INHIBIN B concentration

The group of women patients was divided according to the last miscarriages date into three groups, the first group the last miscarriage one month before to four months, the second group from five months to

nine months, and the third group more than nine months to two years and the relation with the concentration of INHIBIN B for the purpose of knowing their effect on the level of these hormone, the measurement showed insignificantly decreased in the second group (5-9 months) when compared with the first group (1-4 months), as shown in figure (5).

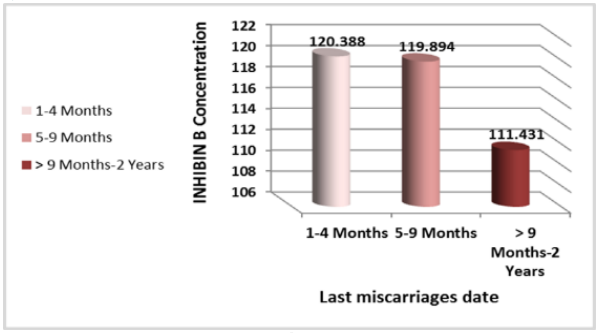

Figure (5): The relation of last miscarriages date with INHIBIN B concentration.

4. Discussion

INHIBIN B does seem to be related to fertility, as low levels of INHIBIN B is associated with impaired ovulation, low pregnancy rates and increased danger of miscarriage [20]. INHIBIN B is an important indicator of ovarian reserve (the ovary's capacity to respond to gonadotropin stimulation) predicts the magnitude of retrievals, and is used to determine Ovarian Hyper-stimulation Syndrome determines gonadotropin dosage for Assisted Reproductive Technologies [21].

According to the presented data show mean of INHIBIN B levels (115.56 ± 10.93pg/ml) in patients for age years and (133.64 ± 10.00pg/ml) in control group, that significantly decreased in patients group (P=0.01), as shown in table (1). Study suggests the amount of INHIBIN B relates with follicular function and oocyte quantity, suggesting that INHIBIN B cell product plays a role in follicular growth [22]. Another study found that the amount of both INHIBIN B proteins released into follicular fluid appears to rise with follicle growth, while concentrations may somewhat fall in the biggest follicles due to strength in a larger fluid volume [23]. INHIBIN B levels decrease before blood FSH levels rise, making it a more sensitive indicator of ovarian age than FSH [24]. INHIBIN B levels were lower in patients using progesterone for polycystic ovarian syndrome [25].

According to the presented data show mean of INHIBIN B levels (117.00± 9.93pg/ml) in patients for age [16, 18-21, 23, 25-29] years and (135.87 ± 6.69pg/ml) in control group, that significantly decreased in patients group (P=0.01), as shown in table (2). INHIBIN B levels are now thought to be the strongest predictor of reproductive potential. In fact, when it comes to determining reproductive potential, INHIBIN B outperforms follicle-stimulating hormone (FSH). There is a recognized negative association between INHIBIN B and FSH [27]. Premature ovarian failure affects 1% of all women and 0.1% of those under the age of 30. Secondary amenorrhea, infertility, and increased gonadotropin levels are all symptoms of this illness. A study similar found that when the ovarian follicular reserve begins to decrease, serum INHIBIN B concentrations decrease [28]. Another study found that when comparing PCOS to normal people, follicular fluid INHIBIN B levels are lower [29].

According to the presented data show mean of INHIBIN B levels (113.39 ± 12.22pg/ml) in patients for age years and (130.92 ± 12.65pg/ml) in control group, that significantly decreased in patients group (P=0.01), as shown in table (3). Increased or decreased hormone levels that are greater or lower than normal levels might affect the ovarian system and lead to PCOS. As a result, health issues associated with advancing age may increase the chance of PCOS in women diagnosed with health problems such as obese, stroke and other conditions, and PCOS may exacerbate these conditions. As a result, early detection and treatment of PCOS patients are critical for reducing health risks in women. Several Iraqi research have found that women in various localities are at risk for PCOS. In our study, we found that INHIBIN B in two groups [16-42] years, it were in the control group higher than patient's women with PCOS. Study agreement with the study that found INHIBIN B secretion was shown to be declining in older women, indicating a decreased ovarian follicular pool [30]. Another study found that Follicular phase INHIBIN B levels were considerably lower in older females [31].

Several studies and researches supported the result of the study, like as Al-Tu'ma et al. [32] this is a common finding among women with the syndrome. Obesity and abdominal fat accumulation, which a high percentage of female suffer from, aggravate the physiological, hormonal, and metabolic symptoms of polycystic ovarian syndrome [32]. Another study Found that the inability of insulin to operate properly is one of the reasons why people with PCOS gain weight or have difficulty losing weight. Obesity can lead to a variety of major health issues. Increased PCOS symptoms include irregular ovarian cycles, and anovulation. Endocrine and metabolic problems may be influenced by BMI. Obesity has been associated to an increase in number of miscarriages, cardiovascular and infertility risk in the development and progression of the syndrome [33]. According with World Health Organization, thin is defined as a BMI of less than 18.5 kg/m², normal weight is defined as a BMI of 18.5 to 24.9 kg/m², overweight is defined as a BMI of 25 to 29.9 kg/m², and obese is defined as a BMI of more than 30 kg/m² [34]. Obesity has been linked to a decrease in ovulatory rates, an increase in the frequency of miscarriages and possibly an increase in infertility associated with PCOS, according to a new study. Anxiety, melancholy, stress, and personal dissatisfaction, all of which are common in women with PCOS, may be exacerbated by a change in body image as a result of weight increase [35].

The diagnosis of PCOS is necessary because it indicates metabolic problems, possible cardiovascular problems, and, most importantly, it interferes directly with these patients' reproductive state [36]. INHIBIN B levels are inversely associated to BMI in polycystic ovarian syndrome. Suggesting that BMI may inhibit INHIBIN B release, and obesity reduces follicle health follicular production of INHIBIN B when compared to the control group.

Early miscarriage is described as a pregnancy loss that happens within the first three months of pregnancy (less than 12 weeks gestation) and affects 1-5% of pregnancies. Late miscarriage happens in the second trimester (12–24 weeks of pregnancy) and uncommon happen, affects in 1–2% of pregnancies [38]. Females with polycystic ovary syndrome are three times more likely than women without PCOS to miscarry in the first trimester of pregnancy [43]. INHIBIN B concentrations were shown to be greater in embryo blood throughout the first trimester, according to research by Muttukrishna et al. [26].The fact that decrease INHIBIN B concentrations indicate ovarian insufficiency in women who had missed miscarriages suggests that it may be possible to use INHIBIN B to predict the viability of an early pregnancy [39].

The most significant risk for miscarriage is a recent spontaneous miscarriage, according to a large body of research on recurrent miscarriage. Because a prior spontaneous miscarriage is the most important predictor of future spontaneous miscarriage, the result of a woman's first pregnancy has far-reaching significance for all future pregnancies [40]. Endocrine problems are thought to be responsible for around 8% to 12% of all occurrences of recurrent miscarriages [41]. Moreover, when a pregnancy is obtained, PCOS patients are more likely to suffer recurrent miscarriages [42]. Several research have investigated the correlation between PCOS and recurrent miscarriages in recent years. Women with recurrent miscarriages have been found to have higher rates of PCOS [44]. In fact, INHIBIN B levels never rise to high levels throughout pregnancy, and their highest levels at birth are similar to their peak mid cycle values a few days after ovulation [21].

Increasing pregnancy period after a miscarriage, tends to enhance birth outcomes in the subsequent pregnancy. In order to improve results, a world health organization (WHO) expert consultation on birth spacing suggests deferring the next pregnancy for at least 6 months after a miscarriage. This advice was based on the findings of a single big Latin American study, which found that miscarriage–pregnancy durations fewer than 6 months were linked to a higher risk of preterm birth [45]. Study in California found that revealed no evidence of negative related with pregnancies soon after a miscarriage [46]. Another study found that, in the setting of recurrent miscarriages, fertility is highest when the delay between pregnancies is at least one per year. This suggests that the appropriate waiting period for a pregnancy is around 6–9 months; a conclusion that has been somewhere else in relation to the time to pregnancy following a miscarriage [47]. After the lady has experienced many pregnancies. Our finding suggest that when the waiting period reaches around 6 to 9 months, there should be knowledge of masculinity factor. Use of nicotine has been linked to an increased risk of miscarriage and infertility [48]. Women's fertility decrease gradually but significantly starting around age 32 and more quickly after age 37. This is mainly due to a decrease in egg production in conjunction with a slight rise in circulating levels of follicle-stimulating hormone and a decrease in INHIBIN B concentrations [49]. The decrease of INHIBIN B levels are a more accurate late predictor of decreased follicle number. As a result, INHIBIN B levels are not suggested to be used as a marker of the ovarian poor response to ovarian stimulation or as a useful biomarker of ovarian reserve [50].

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