THE INVESTIGATION OF THE EFFECTS OF SELENIUM BINDING PROTEIN 1 (SBP-1), ANTI-MULLERIAN HORMONE AND ANTRAL FOLLICLE COUNT ON PREGNANCY IN PRIMARY INFERTILITY PATIENTS

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ABSTRACT Introduction: Selenium binding protein 1 (SBP1) is a cytosolic protein, and a low serum level of SBP1 is a marker for women at risk of ovarian failure and infertility. The aim of this study is to meet the need for a marker that can be used in the clinic as an alternative to anti-Mullerian hormone (AMH) in primary infertile patients. Materials and Methods: A total of 82 women, 63 in the study and 19 in the control groups, were included in the study. On the 2nd or 3rd days of the menstruation, patients were examined with transvaginal sonography (TVS), and the number of antral follicles (AFs) was calculated. Then, laboratory blood samples were taken to measure AMH, SBP1, FSH, LH, E2, prolactin and TSH values. Results: SBP1 levels were similar in the study and control groups. No statistically significant relationship was found between SBP1 and AMH, AFs, BMI, age, pregnancy outcome, FSH, LH, E2 parameters. Measures in which the success of distinguishing individuals from the study group and the control group were significant were found only as age and AMH variables. Conclusions: AMH shows the number of primordial follicles, provides information about the health status of the ovarian tissue, and the serum level of AMH decreases with years, and there is a weak relation with SBP1 and these values, but there is a statistically insignificant relationship, information about ovarian tissue such as AMH; these also suggest that women can use it as an ovarian reserve test regardless of their cycle.

KEYWORDS primary infertility, selenium binding protein 1, anti-mullerian hormone, antral follicle count, pregnancy.

Introduction

Infertility is the clinical condition that defines a couple’s inability to conceive for 12 months and longer when the woman is below 35 years, and 6 months and more when she is over 35 years, despite unprotected and regular sexual intercourse. Couples who have not had a pregnancy before are called primary infertile, and couples who previously had a pregnancy are called secondary infertile [1]. Infertility with normal ovulation, normal tubal patency and uterine cavity, normal semen analysis, and sufficient ovarian reserve is called “unexplained infertility” [2,3]. Unexplained infertility can be diagnosed in up to 15-30% of infertile couples [4]. In the evaluation of infertile couples, the first evaluation including a complete medical history and physical examination, should be performed. Basal hormone levels in infertile couples, especially in menstruation 2nd or 3rd days, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) should be analyzed. Both ovaries and antral follicles (AFs) should be evaluated by transvaginal sonography (TVS) in patients. By determining the serum anti-Mullerian hormone (AMH) value the progesterone level on the 22nd day of
menstruation in the same cycle of the female individual for the couple with the help of the laboratory; the first road map for follow-up and treatment is determined [5].

Anti-Mullerian hormone; is made by granulosa cells in small antral and pre-antral follicles and can be correlated with ovarian reserve, ovarian function, regular menstrual cycle, in practice [6,7]. In infertile women, AMH is a predictive tool for ovarian response to stimulation of gonadotropin hormone, but it does not reflect the possibility of pregnancy [8,9]. Serum AMH levels are inversely proportional to age, but it remains relatively constant throughout the menstrual cycle and can be measured at any time [10]. Normal AMH values are between 1-3 ng/ml in women of reproductive age. While low values such as 0.2-0.7 ng/ml predict that ovarian stimulation will be unsuccessful, it still does not provide sufficient information about the possibility of pregnancy [11]. In pregnancy, serum AMH levels are measured low due to suppression of follicle formation. In patients with polycystic ovary syndrome (PCOS), AMH values are found approximately 2-3 times increased. Although it is argued that high body mass index (BMI) decreases AMH levels, it is claimed that the main factor is the age, not obesity. There are discussions about the qualitative benefit of AMH [12].

It was observed that the ovarian reserve predicted with maternal serum AMH values correlated closely with the number of AFs detected in the early follicular phase with TVS. However, the correlation was valid in AFs counts with follicles classified as small and medium; in cases where the follicles over 6 mm are dominant, it is claimed that the correlation between the number of AFs and AMH has disappeared [13]. In studies conducted, it was observed that there was a correlation with the AFs count and the number of primordial follicles [14]. The AFs count also decreases in a woman approaching menopause and in individuals with poor ovarian reserve. However, the reliability of the number of AFs in evaluating the ovarian reserve is very low. For this reason, it is not used alone in evaluating the ovarian reserve and it is correlated with other parameters.

Selenium binding protein 1 (SBP1) is a cytosolic protein which partially characterized in 1989. SBP1 is different from other proteins containing selenium in selenium binding mode. For example, while glutathione peroxidase, thioredoxin reductase, and other enzymes are proteins containing selenocysteine, SBP1 is assumed to be common. In cases of infertility, serious ovarian cancer, endometriosis, anovulation, PCOS, premature ovarian failure and other conditions where ovarian tissue is affected, auto-antibodies against SBP1 are seen and serum levels of SBP1 decrease [15]. Low serum level of SBP1 is a marker for women at risk of ovarian failure. In addition, it was observed that the amount of selenium decreased in primary oocyte fluid content in women whose cause was not explained and received IVF treatment [16]. That’s why, it is claimed that SBP1 serum levels can be used in unexplained infertility [17-19].

The aim of this study is to meet the need for a marker that can be used in the clinic as an alternative to AMH in primary infertile patients. While the AFs count is used in the evaluation of the ovaries or the course of treatment in women receiving infertility treatment, the evaluation of SBP1 serum levels as a correlation with AMH or as an alternative marker has been the subject of this research.

**Materials and Methods**

**Study design and participant population**

In this study, participants were selected from the patients who applied to our IVF Center between 05.2018 and 05.2019. A total of 82 women, 63 in the study group and 19 in the control group, were included in the study. In both groups, women between the ages of 20 and 40 were selected.

**Inclusion criteria**

*As the study group;* female patients; who has not been able to get pregnant even before, without a tubal or uterine disorder in hysterosalpingography, and whose sperm test were evaluated normal, who had no problem in the evaluation of the hypothalamus-pituitary-ovarian hormonal axis, did not undergo ovarian surgery previously, no thyroid dysfunction, and who do not take anti-inflammatory drugs, were included in the study. As a control group, women who had a pregnancy before and brought it to term and gave birth to a live baby even once, who did not have a chronic ovarian disease and did not undergo ovarian surgery, whose ovaries were not removed for any reason, who did not receive anti-inflammatory treatment, who did not have thyroid disease, who women with a normal hormone test on 2nd or 3rd days of menstruation, were included in the study.

**Data collection**

Personal information of the patients was recorded in their first application at the clinic. Physical examinations of patients were performed and menstrual periods, patients and families’ genetic histories, and BMIs were recorded. On the 2nd or 3rd days of the menstrual period, patients were examined with their TVS probe (7.2 MHz, Toshiba Xario 100, Japan) in the outpatient clinic while in the lithotomy position. Then, laboratory blood samples were taken to measure AMH, SBP1, FSH, LH, E2, prolactin and TSH values. The samples taken for AMH, SBP1 was centrifuged within 10 minutes and their serums were stored at -80 °C. Stored serums were studied within two months. The patients were followed up with βhCG values during the clinical follow-up and after treatment. As a result of (+) βhCG values, pregnancy was observed in 23 patients in the primary infertile patient group and 2 patients in the control group.

**Ethical permission**

Ethics committee approval was obtained from the Clinical Research Ethics Committee (E-18-2056) for the study. Each patient was informed in detail and informed consent forms were received. The ethical rules the determined were followed at each stage of the study.

**Statistical Analysis**

The statistical analyses were performed with SPSS 22 statistical software. Along with descriptive statistics, Spearman correlation analysis, Mann-Whitney U test, Kruskal Wallis test and Chi-square test were used to correlate analysis and the predictive values of the parameters were compared. Besides, independent parameters such as AMH, SBP1, age, BMI, duration of infertility were also examined by ROC analysis. P value <0.05 was considered significant.
Table 1 Relationship between selenium binding protein 1 and AMH and BMI values

|                   | Study group | Control group |
|-------------------|-------------|---------------|
| **SBP1 and AMH**  | r           | -0.373        |
|                   | P           | 0.326         |
|                   | n           | 63            |
| **SBP1 and BMI**  | r           | -0.168        |
|                   | P           | 0.188         |
|                   | n           | 63            |

Test analysis: Spearman Correlation coefficient.

Table 2 Comparison between selenium binding protein 1 values according to the evaluation of the first basal antral follicles of the individuals in the Study and Control groups and comparison of the selenium binding protein 1 measurements according to the groups.

| n   | Mean | SD  | U        | P   |
|-----|------|-----|----------|-----|
| **Study group SBP1 levels** |      |     |          |     |
| AF  | 29   | 6.47| 2.60     | 449.50 | 0.549 |
| PCO | 34   | 6.85| 2.64     |       |       |
| **Control group SBP1 levels** |      |     |          |     |
| AF  | 13   | 6.62| 2.46     | 35.00  | 0.726 |
| PCO | 6    | 5.95| 2.15     |       |       |
| **SBP1 levels** |      |     |          |     |
| Study | 63   | 6.68| 2.61     | 543.00 | 0.542 |
| Control | 19   | 6.41| 2.33     |       |       |

Test analysis: Mann Whitney U test. SD, standard deviation.

Table 3 The relationship between selenium binding protein 1 measurements according to pregnancy results of individuals in the study and control groups with a pregnancy result.

| n   | SBP1 level | Chi-square | P   |
|-----|------------|------------|-----|
| **Study group** |      |            |     |
| Abortion | 5     | 7.14       | 3.18 | 1.724 | 0.632 |
| Live pregnancy | 8     | 6.26       | 2.47 |       |       |
| Pregnancy ext. center follow-up | 1     | 9.01       |       |       |       |
| In the term-live birth | 8     | 7.08       | 2.34 |       |       |
| **Control groups** |      |            |     |
| Live pregnancy | 2     | 3.77       | 1.38 |       |       |

Test analysis: Kruskal Wallis testi. SD, standard deviation.

Table 4 Area Under the Curve

| Variables | Area | SD  | Severity Level | Confidence Interval 95% |
|-----------|------|-----|----------------|-------------------------|
|           |      |     |                | Lower Limit | Upper Limit |
| Age       | 0.244| 0.063| 0.001*        | 0.120       | 0.367       |
| BMI       | 0.456| 0.071| 0.560         | 0.316       | 0.596       |
| SBP1      | 0.546| 0.077| 0.542         | 0.395       | 0.698       |
| AMH       | 0.635| 0.073| 0.042*        | 0.512       | 0.797       |
| AFS count | 0.638| 0.073| 0.070         | 0.494       | 0.782       |
| FSH       | 0.421| 0.072| 0.296         | 0.280       | 0.561       |
Results

The relationship between SBP1 and AMH measurements is shown in Table 1. A positive and weak relationship between SBP1 and AMH values in the study group (r=0.126); in the control group, a negative and moderate (r=-0.337) relationship was observed and both values were not statistically significant (p>0.05). A negative and weak relationship between SBP1 measurements and BMI measurements in the study group (r=-0.168); a positive weak relationship (r=0.240) was found in the control group and both values were not statistically significant (p>0.05).

In the study group, there was no significant difference between SBP1 values according to whether the individuals were the first basal AFs or not (p>0.05). Similarly, there was no statistically significant difference in the same parameters in the control group. (p>0.05). Besides, there was no significant difference between the SBP1 values according to the groups (P> 0.05). (Table 2)

No significant differences were found between SBP1 measurements according to age groups of study and control groups (p>0.05).

There was no significant difference between the SBP1 measurements according to the pregnancy results of the individuals in the study group (p> 0.05).

There was no significant difference between the SBP1 measurements according to the pregnancy results of the individuals in the study group (p> 0.05). Since only live pregnancy was observed in two cases in the control group, comparisons by the SBP1 level could not be made. (Table 3)

A positive and weak relationship (r=0.208) was found between the SBP1 and FSH measurements of the individuals in the study group, and this relationship was not significant (p> 0.05). Also, there was no relationship between SBP1 and LH and E2 measurements. In the control group, a negative and weak correlation (r=-0.216) was found between the SBP1 and FSH values and it was not significant (p>0.05). A negative and weak relationship (r=-0.214) was found between SBP1 and LH values and was not significant (p> 0.05). There was no linear relationship between SBP1 and E2 measurements.

Table 4 showed whether age, BMI, SBP1, AMH, AFs count, and FSH measurements were determinative in distinguishing participants in the study and control groups. Age and AMH variables were found to be significant in the success of distinguishing individuals from the study group and the control group (p <0.05). In other words, the power of age and AMH measurements to identify individuals in the study and control groups were strong.

The ROC curve for AMH measurements is shown in Figure 1-A. The area under the ROC curve for age measurements was 0.25 unit-square. The probability of an individual in the study group to have a more suspicious (+) test result than an individual in the control group was 0.25. Again, for significant AMH measurements, the area under the ROC curve was 0.66 unit-square. In other words, the probability of an individual in the study group to have a more suspicious (+) test result than an individual in the control group was 0.66. The cut-off value of AMH measurements was 2.81 in diagnosing individuals in the study or control group. For this cut-off value, sensitivity was calculated as 0.75 and specificity as 0.474.

The cut-off value of "age" measurements in the study or control group was 21.50. The sensitivity was 0.91 and the specificity was 0.947 for the 21.50 cut-off value. In other words, the rate of diagnosis of individuals in the study group was 91%, with the "Age" measurement less than 21.50. So, the specificity value from the 0.947 transactions was 0.053. In other words, the rate of identification of individuals with the "Age" measurement less than 21.50 was 5% in the control group. (Figure 1-B)

Since the BMI, SBP1, AFC and FSH measurements were not determinant in separating individuals in the study and control groups, only ROC curve values were included. (Figure 2)

Discussion

In our study; FSH, LH, E2, TSH, prolactin, AMH and SBP1 values were examined on the 2nd and 3rd days of menstruation in primary infertile patients. Besides, with TVS, on the 2nd and 3rd days of menstruation, ovaries were evaluated, and the number of AFs was calculated. In a one-year follow-up, each patient’s pregnancies were followed up with βhCG values. According to our results, SBP1 levels were similar in the study and control groups (p> 0.05). No statistically significant relationship was found between SBP1 and AMH, AFs numbers, BMI, age, pregnancy outcome, FSH, LH, E2 parameters. Measures in which the success of distinguishing individuals from the study group and
Specific auto-antigens such as HSP90 [20], enolase [21], and SBP1 [22] were reported in premature ovarian failure and infertility. In an immunoassay study, Yu-Rice et al. [23] reported that the mean optical density (0.72; \(p=0.04\)) and the proportion of sera positive (30%; \(p=0.03\)) for anti-SBP1 were significantly higher in infertility compared to normal controls. Among women with infertility, the mean optical density value for anti-SBP1 was significantly higher in women with ovulatory dysfunction (\(p<0.0001\)), unexplained infertility (\(p=0.01\)), and premature ovarian failure (\(p=0.03\)) compared with healthy controls. The proportion of anti-SBP1 positive sera was significantly higher in women with ovulatory dysfunction (50.0%; \(p=0.007\)), unexplained infertility (24.3%; \(p=0.02\)) and premature ovarian failure (28.0%; \(p=0.02\)), compared to control. However, in our study, no significant difference was found between the SBP1 measurements of sera (sera) in the primary infertile group and the control group (\(p>0.05\)).

AMH plays a central role in paracrine control of ovarian steroidogenesis and folliculogenesis, and this hormone plays a major role in the pathophysiology of anovulation, PCOS, low ovarian reserve, and primary ovarian failure [24]. In our study, a positive and weak relationship was found between SBP1 and AMH values in the primary infertile patient group, and this relationship was not statistically significant (\(r=0.126; p>0.05\)). These results suggest that SBP1 cannot be used like AMH in order to show the number of primordial follicles in ovaries. Also, we could not find any study on this subject. It was found that the relationship between the ovarian reserve and SBP1 levels was investigated very little, only auto-antibodies developing against SBP1 decreased serum levels of this protein and also this situation was not examined in terms of infertility. It was also observed that the association of serum SBP1 and AMH was not examined in terms of female infertility. However, in a study related to other parameters, no correlation was found between anti-SBP1 and CA125 and anti-p53 in infertility (correlation coefficient = 0.07; \(p=0.59\) and correlation coefficient = 0.25; \(p=0.07\), respectively). Besides, anti-SBP1 discriminated infertility (premature ovarian failure, unexplained infertility, ovulatory dysfunction, and endometriosis) from healthy controls with an AUC of 0.7 (\(p=0.001\)) [23]. Considering the infertility process and its causes, the fact that SBP1 and AMH levels are correlated even if weak, suggests that this study should be performed with the more patient population. As a new usable parameter related to the viability and health of ovarian tissue that is not affected by external parameters, SBP1 levels require more detailed evaluations.

In the primary infertile group of patients, there was no significant difference between the SBP1 measurements according to whether the individuals had the first basal AFs or not (\(p>0.05\)). A negative and weak correlation was found between SBP1 measurements and BMI measurements in the primary infertile patient group (\(r=-0.168; p>0.05\)). This finding confirms the information that the increase in BMI, which is an already known phenomenon, causes a decrease in ovarian follicle quality. The inverse proportion found in this study should also be evaluated by increasing the size of the session to be studied and the size of the study group (universe). No significant correlation was found between the SBP1 and FSH, LH and E2 measurements of the individuals in the primary infertile patient group. Negative and weak relationships were found between SBP1 and FSH and LH values of the individuals in the control group and the relationships were not statistically significant (\(r=-0.216\) and \(r=-0.214; p>0.05\), respectively). Ovarian reserve; shows the reproductive potential of ovaries, represents the number of oocytes available for fertilization and can be evaluated by blood tests or TVS [25]. Although there are no strict criteria for decreased ovarian reserve, the following values can be considered consistent with decreasing ovarian reserve: AMH value less than 1 ng/ml, AFs number less than 5-7, FSH value greater than 10 IU/L. Poor response to in-vitro fertilization stimulation (Less than four oocytes during oocyte uptake [26]. The ovarian reserve can be evaluated by measuring E2 and FSH between the 2-5-day cycles of menstruation. FSH values greater than 10 IU/L are associated with a less robust response to ovarian stimulation [27]. Basal E2 levels should typically be less than 60-80 pg/ml; high E2 levels can have a suppressive effect on FSH levels and indicate that ovarian reserve decreases. Infertility is a problem that brings both social and health problems. Besides the known sociological problems, for example, the rate of ovarian cancer is significantly increased in women with infertility compared to the populations [28,29]. Anti-SBP1 in women with infertility related to ovarian insufficiency may be a measurable indicator of risk for ovarian cancer [23].

Conclusions

As a result of this study; there was a negative and moderate relationship between SBP1 and AMH levels in the control group (\(r=-0.337; p>0.05\)). Although there was a low relationship in the primary infertile group of patients who had not conceived before, this correlation was considered as a non-significant relationship since the control group had previously experienced pregnancy. In other words, AMH shows the number of primordial follicles in the ovarian tissue, provides information about the health status of the ovarian tissue, and the serum level of AMH decreases with years, and there is a weak relation with SBP1 and these values, but there is a statistically insignificant relationship, information about ovarian tissue such as AMH; these also suggest that women can use it as an ovarian reserve test regardless of their cycle. However, in the control group, that is, in the previously conceived population, there is a negative relationship between FSH and LH and SBP1 and SBP1 is high in individuals with low FSH and LH indicating that the healthy ovarian tissue has a healthy follicle amount and a pregnancy in its history. Serum SBP1 is thought to be high because of inflammation, autoimmunity, or any pathological process in the ovarian tissue that is considered to be or is likely to be normal. It was thought that this situation should be brought to a clearer and clinically usable stage by working in multiple centers.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by no one.

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