NEOPTERIN AND RECURRENT SPONTANEOUS ABORTİON (RSA): THE EFFECT OF CELLULAR IMMUNE SYSTEM ACTIVATION ON SUBSEQUENT PREGNANCY

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Abstract:

Background: Recurrent miscarriages are common complications in pregnancy. Neopterin is one of the immunologic biomarkers of several diseases related to activation of the cellular immune system. RSA is associated with immune system related abnormalities.

Objectives: We aimed to investigate the effectiveness of neopterin levels in the early diagnosis of RSA.

Methods: Eighty RSA patients and forty-three healthy controls were included in the study. The neopterin concentrations were determined by the enzyme-linked immunosorbent assay (ELISA) method. For the statistical analysis, Mann-Whitney U test and Pearson correlation test were used the; $p<0.05$ was considered statistically significant.

Results: Serum mean neopterin levels were 16.47±0.095 nmol/L in RSA group and 6.14±0.041 nmol/L in control group, respectively. Compared to the control group, a statistically significant increase (p=0.0183) in the serum neopterin levels of the patients was observed. There was a negative correlation between serum neopterin level and age in both the control (R=0.0774, p=0.6236) and RSA groups (R=0.1415, p=0.2089). However, this correlation was not statistically significant (p>0.05).

Conclusions: With overstimulation of interferon-γ (INF-γ) during pregnancy, the production of neopterin increases by monocytes/macrophages. The measurement of neopterin levels in the serum contributes to the early diagnosis of pregnancy losses.

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Introduction

Two types of pregnancy loss have been described, namely sporadic and recurrent pregnancy loss. Recurrent pregnancy loss is observed at a rate of about 1%, while the rate of sporadic pregnancy loss is reported to be higher (25 to 50%) and is mostly observed to develop in association with chromosome abnormalities during advanced age pregnancies (Rai & Regan, 2006). RSA is one of the most common complications of pregnancy and adversely affects many pregnant women (Smith & Cowchock, 1988).RSA is defined as the occurrence of three or more failed pregnancies between the last day of menses and 20th week of gestation, or fetal rejection less than 500 g of fetal body weight (Rai & Regan, 2006; Strobino & Warburton, 1995; Kwak-Kim et al., 2000).

Factors associated with pregnancy loss include disorders related to genetic, hormonal, metabolic, or uterine anatomy, infections, environmental and occupational exposure (lead, mercury, ethylene, oxide, radiation), and personal habits (alcohol consumption and cigarette smoking), thrombophilia or the immune system (Kwak-Kim et al., 2000; Pandey et al., 2004). Immunological mechanisms are suggested to play a role as a possible explanation for pregnancy losses, in the absence of identification of any of these etiologies (Bellingard et al., 1995).

Neopterin is secreted from T-lymphocytes through the GTP (guanosine triphosphate) cyclohydrolase I enzyme by overexpression of IFN-γ from activated monocytes, macrophages, and dendritic cells. The activity of this enzyme is greatly increased by IFN-γ and other cytokines (Milich et al., 1995; Fuchs et al., 1992; Muller et al., 1991).

Neopterin Levels in Subsequent Pregnancy

Study Groups

A total of eighty women with a history of at least three abortions of unknown etiology which occurred during the first trimester were included in the study. The control group consisted of forty three healthy women. The mean age of the RSA group was 29.11±0.07 (year), whereas that of the control group was 31.18±0.19 (year).

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All individuals in the control group were thoroughly scrutinized about their health status. This study was approved by the Institutional Ethics Committee and conducted in accordance with the principles of the Declaration of Helsinki. A written informed consent was obtained from each participant.

Neopterin Measurement

Blood samples were collected into 10 cc biochemistry tubes for biochemical and histological tests required for routine follow-up blood sampling. Blood samples were collected into heparin-containing vacuum tubes and centrifuged at 3.500 rpm. Supernatants were collected and stored at -20 ^{o}C. Serum neopterin levels were measured using the ELISA (DRG Diagnostics GmbH, Germany) method. Neopterin concentrations were expressed in nmol/L.

Statistical Analysis

Statistical analysis was performed using the SPSS version 11.50 software. Descriptive data were expressed in mean±standard error (SE).The Mann-Whitney U test was used to compare two independent groups, whereas the Pearson correlation test was used to analyze possible relationships between the variables. A *P* value of <0.05 was considered statistically significant.

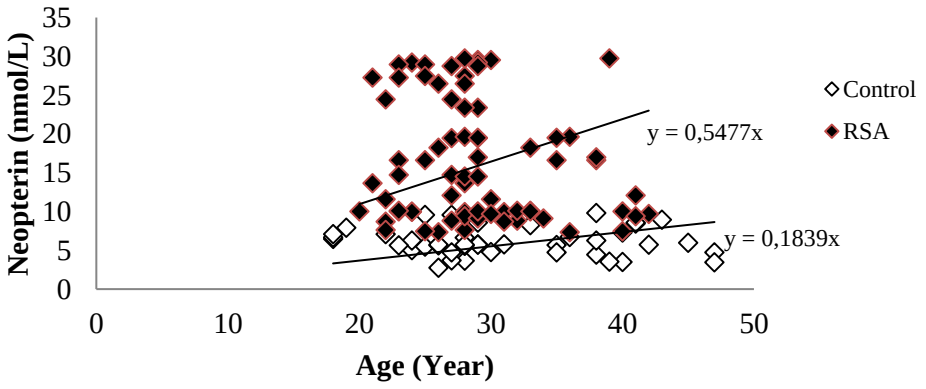
Discussion

The elevated serum concentrations of neopterin have been demonstrated in various infections (malaria, measles and septic shock), chronic inflammatory diseases (Crohn disease, ulcerative colitis) and autoimmune disorders (rheumatoid arthritis, thyroiditis), cardiovascular diseases, malignancies and organ transplantations (Muller et al., 1991; Fuchs et al., 1993; Eisenhut, 2013; Yanchun&Zhidong, 2011). Neopterin is a sensitive indicator of cell-mediated immune activation (Eisenhut, 2013; Yanchun&Zhidong, 2011; Huber et al., 1984). Neopterin levels can be used as an indicator in the assessment of immunogenic stimulations induced by the fetus and placenta during pregnancy since its production reflects cellular immune response (Munn et al., 1998).

The mean serum neopterin level was 16.47±0.095 nmol/L in the women with RSA and6.14±0.041 nmol/L in the control group, indicating statistically significantly higher levels in the women with a history of RSA (p=0.0182).

In addition, we found a negative correlation between the serum neopterin levels and age both in the control group (R=0.0774, p=0.6236) and in the RSA group (R=0.1415, p=0.2089) (Figure 1).However, this correlation was not statistically significant (p>0.05).

Figure 1. The distribution of neopterin concentrations according to the age.



Source: Author

A healthy pregnancy is characterized by depression of cell-mediated immune response in addition to an increase in humoral immune response (Kaleli et al., 2005). The production of Th2 cytokines by decidual T-cells contributes to the progress of pregnancy; however, the excessive increase in Th1 response poses a risk for the survival of the fetus. The predominance of Th1 may be associated with pathological conditions such as RSA and preeclampsia (Kaleli et al., 2005; Lin et al., 1993).

Women with recurrent pregnancy losses and/or inadequate implantation have been reported to possess significantly increased peripheral blood Th1 cells compared to normal fertile women. Increased

proinflammatory cytokines have been suggested to play an important role in recurrent pregnancy losses (Kwak-Kim et al., 2000). Excessive activation of Th1 cytokines and natural killer (NK) cells has been reported to be the most important alloimmune cause of RSA (Pandey et al., 2004). NK cells in the uterine mucosa have been reported to contribute to the cytokine response at the maternal-fetal interface. This cytokine response is generally caused either by type Th1 (interleukin 2, INF-γ and tumor necrosis factor alpha (TNF-α) production) or type Th2 (interleukin 4, 6 and 10) cells. A normal pregnancy may be the result of Th1-type cytokine response. On the other hand, women with a history of RSA present with a predominantly Th1-type response during embryonic implantation and during pregnancy (Rai & Regan, 2006; Piccinni et al., 1998).

Many studies have been conducted which suggest that complications of pregnancy which lead to disorders of placentation such as preeclampsia and fetal growth retardation are associated with increased decidual cellular immunity (Erkenekli et al., 2015; Bartha &Comino-Delgado, 1999). About 20% of pregnancies result in spontaneous abortion. About 60% of spontaneous abortions are due to genetic, infectious, hormonal and immunological factors. Under certain conditions, the immune tolerance mechanism may be impaired and the fetus immunologically rejected. The effect of immune mechanisms is also associated with the gestational period during which the abortion occurred. During preimplantation and until the end of implantation, cell-mediated immunity is said to be responsible for early abortion. Immunocompetent decidual cells or cytokines have been reported to be responsible for these immunological mechanisms (Giacomucci et al., 1994). 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. As a result, during RSA, paternal lymphocytes immunotherapy is thought to have a beneficial effect on the provision of specific and non-specific T-cell suppression. The risk of the next pregnancy loss is approximately 24% in women with a history of two abortions, 30% after the third, and 40% after four abortions (Regan et al., 1989).There is a possibility of RSA in women with previous live births. History of previous delivery is an independently associated indicator of the result of the next birth. 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 increases with the decrease in the number and quality of oocyte present. The risk is higher particularly in women over the age of 35 (Green-top Guideline, 2011).

Women with RSA were found to have local and systemic immunological changes when compared to women with normal pregnancy. Implementation of immunological tests to these patients would be beneficial during the appropriate period of pregnancy (Pandey et al., 2004; Magid et al., 1998).

Conclusion

In conclusion, evaluation of neopterin levels with routine clinical tests during pregnancy would contribute to the prognosis. The measurement of neopterin levels in body fluids using the ELISA method is rare in the clinical practice. The addition of neopterin measurement to routine clinical laboratory practice may contribute to early diagnosis of diseases.

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