

THE VALUE OF VITAMIN D SERUM, INTERLEUKIN-6, INTERLEUKIN-10 IN POST ABORTION OF WOMEN IN SAMARRA CITY

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ABSTRACT : The samples of this study were collected in Samarra General Hospital from 20/9/2018 to 1/2/2019. The study included 40 blood samples from women with a complete abortion that confirming by the medical staff in the hospital, abortion was in first or second Pregnancy's trimester and 20 blood samples from healthy women with normal pregnancy in the same periods of pregnancy. Women in both groups had an age range of (15-50) years. The study designed to evaluated the effect of a number of biochemical variables related to abortion. Thus, the results were: Increase abortion that caused by hormonal and environmental factors. It was shown significant decrease in the rate of vitamin D concentration with all groups studied, in addition to the significant differences between the secondary groups of the age and the period of pregnancy groups. In addition, we did not notice significant differences between the secondary groups of age. There were differences in the rate of concentration of hormone between periods of pregnancy groups. The study did not detect significant differences with the rate of interleukin-6 concentration for the major groups and secondary groups. The study shown significant decrease in the rate of interleukin-10 concentration in the group of aborted women compare to women with normal pregnancy groups. In addition, there were differences in the concentration of this interleukin between the secondary groups of the age and the period of pregnancy groups.

Key words : Vitamin D, interleukin-6, interleukin-10, Samarra.

INTRODUCTION

Recently, spontaneous abortion (SA) is one of the most problems that women face infertility, effects of those problems were psychological and physiological. (SA) Occurs before 20 weeks' of gestation or when fetus weighs <500 g (Pereza *et al*, 2017). Etiology of (SA) that associate with our study are immunologic mechanisms, maternal infections and possibly environmental exposures (*e.g.* irradiation, smoking, vitamins deficiency, certain drugs) (Kaur and Gupta, 2016). Complete abortion is total, spontaneous rupture of fetus and placenta (Wasson *et al*, 2019). In this study, we chose vitamin D, interleukin-6 and interleukin-10 to assess the effects of abortion.

Vitamin D, a steroid hormone, is well known to be involved in calcium-phosphate homeostasis and bone metabolism (Ota *et al*, 2014). Vitamin D has become increasingly recognized as a pluripotent regulator of biological functions. Although, vitamin D insufficiency is increasingly recognized as a health problem across the world (Liu *et al*, 2011), impaired vitamin D status during gestation is associated with poor skeletal growth in

childhood (Tamblyn *et al*, 2015). However, low maternal levels of the major circulating form of vitamin D, 25-hydroxyvitamin D₃ (25OHD₃), have also been linked to adverse outcomes in pregnancy such as pre-eclampsia, a disorder involving dysregulated placental vascularization that affects up to 10% of pregnancies (Lagishetty *et al*, 2011). Expression of vitamin D receptor (VDR) for the active form of vitamin D, as well as the 1 α -hydroxylase enzyme has been reported for various tissues that can be broadly termed 'barrier sites' (Townsend *et al*, 2005), the presence of VDR in the placenta suggests that vitamin D functions in tissue-specific fashion at the fetal-maternal interface (Shahrokhi *et al*, 2015). One possible explanation is that 1,25(OH)₂D acts as a regulator of placental calcium transport, but a placental immune-modulatory function has also been proposed (Liu and Hewison, 2012). Moreover, the rapid induction of VDR early in pregnancy (Zehnder *et al*, 2002) suggests that vitamin D may play a more fundamental role in the process of conception, implantation and development of the placenta itself (Urrutia *et al*, 2012). In addition to this physiological function, vitamin D modulates the immune system, thus vitamin D deficiency/insufficiency could increase the risk of many chronic

diseases involving immunological dysfunction (Harmon *et al*, 2016).

The human reproductive process is regulated by the immune system, the endometrial lymphocytes play some roles in the maintenance of pregnancy via immune mediators such as cytokines (Chaouat *et al*, 2002). The T helper 1 (Th1) type of cells which cytokine secretion such as IFN- γ , IL-2 and TNF. On the other hand, Th2 cytokines secretions such as IL-10, IL-4, IL-6 and IL-13 have been associated with successful pregnancy (Abdullah and Mahdi, 2013). Different studies have shown that the role of balance of Th1/Th2 immunity play a major role in reproductive phenomena, in which Th2-type dominant response has been associated with normal pregnancy, whereas the Th1-type response has been related to pregnancy failure (Liu *et al*, 2015).

Interleukin-6 is a pleiotropic cytokine produced in response to tissue damage and infections (Tanaka *et al*, 2014). Multiple cell types including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells and T & B cells are associated with the production of this cytokine (Mauer *et al*, 2015). The biological consequences of IL-6 production have been associated with both pro- and anti-inflammatory effects. So far, the role of IL-6 has not been determined at any stage of pregnancy (Scheller *et al*, 2011).

IL-10 is a major immunosuppressive cytokine. It is a critical component in the maintenance of the fine balance between swift and potent immune responses against invading pathogens and the control of detrimental pathological injury (Kalkunte *et al*, 2011). Almost all cells of the innate and adaptive arms of the immune system can produce IL-10, including macrophages, mast cells, NK cells, eosinophils, neutrophils, B cells, Th2 and Th17 cells (Trinchieri, 2007). The major role of IL-10 is to limit the extent of the activation of both the innate and the adaptive immune cells to maintain a homeostatic state (Saraiva *et al*, 2010). IL-10 regulates produce or differentiation (or both) of B cells, NK cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes and endothelial cells (Jenkins *et al*, 2000). IL-10, produced by cytotrophoblasts and decidual T cells, protect the fetal-placental interface by reducing the cytokine secretions of Th1 cells and macrophages (Brogin *et al*, 2012). IL-10 was proposed to be a factor that might protect the semi-allogeneic fetus from maternal allo-recognition and rejection by driving the maternal (both local and systemic) immune reaction toward a Th2-type immune response (Moore *et al*, 2001), IL-10 inhibits pro-inflammatory cytokines production including IL-1 β , TNF-

α and IFN- γ , therefore prevents the development of Th1-type immune reactions deleterious for the maintenance of pregnancy.

MATERIALS AND METHODS

The current study included 60 women with spontaneous abortion (mean of ages is 27.5, ranged from 15 to 45 years) at Consulting Clinic of Samarra General hospital, Salahaddin, over the period from September to February 2019. Samples of control group were collected from 20 pregnant women (They have never had an abortion). Major groups were also subdivided according to women age (15-25, 26-35, 36-45) and gestational month (1-2, 3-4, 5-6). From each participant, venous blood (5 ml) was collected in gel tubes. Separated serum were divided into many aliquots in Eppendorf tubes and kept at -20°C till the tests of parameters were performed. Enzyme-linked immune-sorbent assays (ELISA) were used to evaluate serum levels to each of vitamin D, IL-6 and IL-10.

Statistical analysis

Raw data were analyzed using software of SPSS program (version 20). Student's F-test was used to compare means of parameters between cases and control. The totals were divided into age groups and months of pregnancy groups. Levels of measured factors expressed as (Mean \pm Standard error). Significance in all tests was set at 0.05 ($P \leq 0.05$).

RESULTS

Statistical analysis of the obtained results showed important output for parameters with abortion. Regarding vitamin D, serum levels showed significant dropping, $p \leq 0.05$ compared to control group. There was also a variation in levels of the vitamin for secondary categories of women age and gestational months (Table 1). Moreover, IL-6 showed non-significant variation $P = ns$ compared to control group. There was also no variation in levels of the interleukin for secondary categories of women age and gestational months (Table 2). On the other hand, levels of IL-10 showed significant decrease, $P \leq 0.05$ compared to control group. There was also a variation in levels of the interleukin for secondary categories of women age and gestational months (Table 3).

DISCUSSION

The National Academy of Medicine identified vitamin D normal value about 12ng/mL (Ross, 2011). In this study, the vitamin level was low in all women. We were agreed with the study of Ota *et al* (2014), who said that a high proportion of women with recurrent miscarriages had

Table 1 : The values of vitamin D levels for groups of patients and control.

Vitamin D	Patients	Control	P-value
	4.034±0.759	6.05±1.63	0.0002**
Age	15-25	26-35	36-50
Patients	4.083 ± 0.851c	3.969 ± 0.688c	4.025 ± 0.698c
Control	6.795 ± 1.91a	5.417 ± 1.358b	6.215 ± 0.236ab
Months	1-2	3-4	5-6
Patients	4.063 ± 0.774d	3.937 ± 0.737d	4.218 ± 0.846d
Control	5.155 ± 0.8c	5.952 ± 1.719b	7.029 ± 1.59a

Table 2 : The values of IL-6 levels for groups of patients and control

Interleukin-6	Patients	Control	P-value
	18.29±4.51	20.09±5.95	0.242ns
Age	15-25	26-35	36-50
Patients	18.66 ± 4.77a	17.47 ± 4.37a	18.97 ± 4.37a
Control	20.98 ± 4.42a	20.98 ± 7.12a	18.37 ± 4.02a
Months	1-2	3-4	5-6
Patients	16.73 ± 4.3a	18.6 ± 4.92a	20.09 ± 3.31a
Control	22.66 ± 4.45a	18.91 ± 6.31a	21.99 ± 5.86a

Table 3 : The values of IL-10 levels for groups of patients and control.

Interleukin-10	Patients	Control	P-value
	30.42±7.93	38.47±8.12	0.001**
Age	15-25	26-35	36-50
Patients	32.87 ± 8.86bc	28.11 ± 6.99c	27.62 ± 4.07c
Control	42.29 ± 11.0a	35.95 ± 4.78b	35.87 ± 2.33b
Months	1-2	3-4	5-6
Patients	31.48 ± 8.59bc	29.58 ± 8.37c	30.67 ± 6.28c
Control	43.99 ± 9.56a	36.01 ± 7.58b	42.35 ± 7.03a

Note : letters mean there were significant difference among groups

vitamin D deficiency and low levels of vitamin D are associated with an increased risk of abortion in the first trimester of pregnancy. However, the accurate effect of vitamin D on pregnancy results are still unclear (Andersen *et al*, 2015). In recent years, the role of vitamin D in the fetal-maternal interface has been increasingly recognized and the studies are being search about that (Tamblyn *et al*, 2015). Because of stability and long half-life of 25 (OH) D, it used as the best indicator of the nutritional status of vitamin D in clinical cases (Binkley *et al*, 2009). Recent studies about the effect of D3 on immune cells have indicated that it works with very low nanomolar concentrations to stimulate regulatory T cells and it is an important regulator for preventing T-cell inflammatory response (Thangamani *et al*, 2015). According to a recent study by Hou *et al* (2016), serum levels of D3 were higher in women with normal pregnancies than women with miscarriage in the first trimester, suggesting that vitamin D deficiency was associated with pregnancy loss. In the study of Li *et al* (2017), they investigated D3 concentrations in trophoblastic tissue and found that D3 concentrations in a group of recurrent miscarriages were

low compared with control group, that suggest changing of the mother's vitamin D status could affect pregnancy outcomes. Due to the need for adequate concentrations of vitamin to fill the increasing need for calcium by the fetus during his development and growth (Pérez-López *et al*, 2015). A common problem in women's fertility ages, low levels of vitamin D that found in the 26-35 age group, especially in the Middle East, which probably due to poor vitamin dietary intake, very little exposure to sunlight due to weather conditions (Samimi *et al*, 2015).

Our results differed with Bakir *et al* (2010), which found that IL-6 increases in recurrent spontaneous abortions than in normal pregnant women. IL-6 is a sensitive parameter that indicate inflammations, IL-6 major function is to contribute to immune responses through lymphocytes, IL-6 intermediate in acute phase proteins synthesis and to amplify the toxic activity of natural killer cells (Sharief *et al*, 2014). Therefore, there was no significant difference in the IL-6 serum levels in this study's women because this study was limited to women with miscarriage without any pathogenic causes of abortion, such as *Toxoplasma gondii*, which cause a

significant increase in interleukin-6 serum levels (El-Hashimi *et al*, 2014). The role of Th-2 cells is known in its anti-inflammatory properties and its role in fetal protection from early pro-inflammatory cytokines but the role of IL-6 in pregnancy remains unclear (Saini *et al*, 2011), although that IL-6 high levels were associated with pregnancy complications such as preterm birth (Pressman *et al*, 2011). There were many studies consider IL-6 was among the proinflammatory cytokines, Elevated IL-6 peripheral blood levels in approximately 30 minutes continue to rise for up to two hours when pathogens exposure, stress, and age-related disorders, this may explain that high IL-6 levels in premature birth or recurrent miscarriages as a result of mom's psychological stress or nervousness (Endrighi *et al*, 2016).

The results of this study were agreed with a study by Bohiltea and Radoi (2014) suggesting that increased interleukin-10 gene expression was associated with successful pregnancy, while lower levels associated with fetal loss. IL-10 is an important anti-inflammatory cytokine especially during the development of the fetus, and IL-10 effectiveness is in inhibiting the function of Th-1 cells and phagocytes (Daher *et al*, 2012). Many transcription factors control the regulation of IL-10 gene expression, and failure IL-10 regulate was linked to cancers, immune diseases, early delivery, abortion, fetal development, and pre-eclampsia (Sowmya *et al*, 2014). The fetal-maternal interface consists of trophoblastic tissue that mixed from embryonic cells with specialized maternal lymphocytes and endothelial cells, the gene expression of IL-10 is found in all these cells in addition to the NK cells and the monocyte cells (Kalkunte *et al*, 2008). Thus, IL-10 preserves the immune balance and helps mothers tolerate the fetus and does not reject him, in addition to supporting his growth and development at different stages of normal pregnancy (Faure *et al*, 2016). In the present study, we notes the high IL-10 levels of women aged 36-50, that may explained by the following: The immune system and the endocrine system undergo profound changes with age, increasing susceptibility to infectious diseases and decreasing the functional capacity of innate immune cells, including phagocytes (Scholz *et al*, 2013), T cells produce more proinflammatory cytokines per contra the body try to immune re-balance by secretion of anti-inflammatory cytokines, including IL-10. Thus, reduction of IL-10 affects negatively of pregnancy. Gene expression of IL-10 in normal human placental tissues is expressed at higher levels during the first and second trimester compared to the last three months (El-Azzamy *et al*, 2017). Cheng and Sharma (2015) confirmed that IL-10 is a critical molecule for the success pregnancy

outcomes at all stages and that reducing IL-10 placental gene expression is associated with many adverse pregnancy complications such as spontaneous abortion, early birth (Garcia-Ruiz *et al*, 2015).

CONCLUSION

The study did not detect significant differences with the rate of interleukin-6 concentration for the major groups and secondary groups. The study shown significant decrease in the rate of interleukin-10 concentration in the group of aborted women compare to women with normal pregnancy groups. In addition, there were differences in the concentration of this interleukin between the secondary groups of the age and the period of pregnancy groups.

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