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Correlation between PCNA expression and Cytokine Imbalance in First-Trimester Miscarriage: A Marker of Endometrial Deficiency

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Abstract:

Background: The proliferation marker PCNA (proliferating cell nuclear antigen) is involved in early pregnancy decasualization. This process is crucial for endometrial development and implantation, and PCNA expression reflects the proliferative activity of DSCs. Immunity and inflammation are also of immense importance in maintaining pregnancy. We aimed in this study to assess the expression of PCNA and its relationship with the main cytokines, IL-10 and TNF-α in first trimester miscarriage.

Methods: Sixty-one women (17–39 years old) were subdivided into 3 groups: group 1 RM (Recurrent miscarriage) (N = 25), group 2 Non-RM (Non-recurrent miscarriage) (N = 15), and group 3 term pregnancy control (N = 21). The expression of PCNA in DSCs was assayed by immunohistochemistry (IHC), and PCNA positive cells were counted using light microscopy. The levels of IL-10 and TNF-α in maternal serum were measured by ELISA.

Results: The mean value of PCNA-positive DSC percentages was significantly less for Group 1 (11.04±2.03) and Group 2 (15.3±2.7) compared with that of Group 3 (24.19±2.65) ($p \leq 0.001$). Both groups 1 and 2 also had a marked increase in TNF-α ($p \leq 0.001$) as well as a marked decrease in IL-10, compared to the control group.

Conclusion: Low expression of PCNA in first-trimester miscarriage and its related to proliferation of decidua cell. A simultaneous reduction of IL-10 and increase of TNF-α is an indication of an imbalance of the immune environment that could lead to pregnancy loss. PCNA and inflammatory cytokines are potential molecular markers for evaluating endometrial receptivity and immunity during the early pregnancy.

Keywords: Proliferating Cell Nuclear Antigen PCNA; Decidual Stromal Cells DSCs; Miscarriage; IL-10; TNF-α.

1. Introduction:

structural integrity of placental tissue is made up of a highly managed cellular turnover, which depends on a delicate balance that involves cell multiplication, differentiation, and death [8]. These occurrences take part in organized cell death by apoptosis [9].

Apoptosis refers to a key process in the control of endometrial growth both in regards to the pathology and the physiology [10]. Furthermore, it's widely recognized that the control of placental cell death is necessary for ordinary pregnancy physiology at implantation because apoptosis is vital for proper invasion of the maturing embryo and tissue remodeling/ reorganization of the maternal decidua [11]. Proliferating Cell Nuclear Antigen or PCNA, is a key protein that switches the cellular survival to programmed death. The PCNA also helps in DNA repair process such as nucleotide excision repair, base excision repair, and mismatch repair.

It's a critical protein for multicellular organisms [12]. Recent studies also, highlighted the significant roles of cytokines in regulating these processes. Tumor necrosis factor-alpha (TNF-α) and other pro-inflammatory cytokines are associated with an up-regulation of apoptosis and a decrease in trophoblast

Spontaneous miscarriage means the unplanned end of a pregnancy at a period when the fetus or embryo cannot survive. In humans, it is defined as below 500g in weight or prior to 20 weeks of gestation. Generally, most spontaneous miscarriage happens before the 16th week; among these, 75 percent occur before the 8th week. [1-2]. The causes of spontaneous miscarriage may be summarized as: infection, chromosomal abnormalities, immunological disorders, hormonal problems, environmental factors, uterine abnormalities, nutritional factors, and incompetent cervix. [1,3]. An important factor for placental development is EVT (Adequate extravillous trophoblast); which is necessary for the survival and growth of the embryo. On the invasion of the trophoblast into the uterus on placenta formation, it's crucial that both migration/invasion and proliferation must take place in an accurately coordinated way, where a nutritional path is put in place between the mother and the embryo. Inadequate trophoblast invasion and migration may lead to defective placentation that is linked to clinical pathological conditions of pregnancy, like fetal growth restriction, spontaneous miscarriage, as well as preeclampsia[4-7]. The maintenance of the functional and

environment necessary for successful pregnancy outcomes [14]. Therefore, our study aims to evaluate the expression of PCNA and its association with the main cytokines, IL-10 and TNF-α in first trimester miscarriage.

invasion., contributing to shallow placental implantation and related pathologies [13].In contrast, the maternal-fetal interface develops an immunological tolerance which relies on anti-inflammatory cytokines such as interleukin-10 (IL-10), promoting healthy trophoblast function and placental development. An imbalance between these cytokines may disrupt the immune

2. Methods:

 The third group (Group-3) was made up of 21 females, (term pregnancy): N=21; their mean age was 23.38 ± 3.5years.

Inclusion Criteria:

- Women aged 17–45 years.
- First-trimester incomplete miscarriage (clinically and histologically confirmed).
- History of recurrent miscarriage (≥3 losses) or non-recurrent miscarriage.
- Women with normal term pregnancies included as controls.

Exclusion Criteria

- Presence of systemic diseases (diabetes, hypertension, autoimmune disorders).

2.1 Tissue Collection & Processing :

The study used 61 females aged 17 to 39 years who visited Maternity & Children Hospital situated in Basrah who had an incomplete first-trimester miscarriage. Also, there were women who had ordinary pregnancy during delivery as from October 2016-May 2017. The subjects were categorized into three groups (1, 2, and 3).

 The first group (Group-1) included 25 females with recurrent miscarriage in the first trimester; their mean age was 25.4 ± 4.8 years.

 The second group (Group-2) included 15 females that had an uncompleted first-trimester miscarriage with a minimum of 3 previous non-recurrent miscarriages (ordinary pregnancies); their mean age was 25.6 ± 3.6 years.

and use for detection IL-10 and TNF-alpha by ELISA.

Evaluation of the Immunostaining

It was conducted with the help of a histopathologist. PCNA-immunopositive cells were observed under a regular light microscope, and the number of positive cells per 100 cells was counted by two different observers. were immunoreactive for PCNA per 100 cells. All cells within 10 demonstrative fields were counted and any nuclear staining was considered positive. The expression of PCNA was computed by counting positive cells that had dark brown granules within their nuclei in a single microscopic field under light microscopy (X400). Results were reported as the fraction of PCNA-positive cells subdivided by the total number of cells (positive and negative) in every field and multiplied by 100. This is written as an equation as follows:

PCNA (%) = (Number of PCNA-positive cells in a field / Total number of cells in the same field) × 100 [15].

Statistical Analysis:

study groups. A vertical bar chart was drawn to represent the expression differences between the groups. *P*-values less than 0.05 were considered to be statistically significant.

- Uterine anomalies (fibroids, septum, malformations).
- Infectious causes of miscarriage (TORCH, bacterial vaginosis, etc.).
- Use of immunosuppressive or hormonal therapy within the last 3 months.

Placental specimens and a three ml blood sample were obtained from all the women with miscarriage at the time of curettage. Also, from women who delivered at term, and fixed in 10 percent formalin. The placental specimens were consistently processed for paraffin embedding and validated by a pathologist. Eosin and hematoxylin were used to stain sections (5 μm) for histological analysis. A minimum of one consecutive part of 3 mm was cut from each tissue block; this was for immunohistochemistry (IHC) method utilizing DAKO cytomation detection kit (USA). Following the manufacturer's instructions, the IHC procedure was conducted. At the same time the serum was separate from blood

For data analysis, we employed SPSS (Statistical Package for the Social Sciences). The data are shown as the mean ± SD. Comparative analysis of PCNA, IL-10, and TNF-α expressions in the three

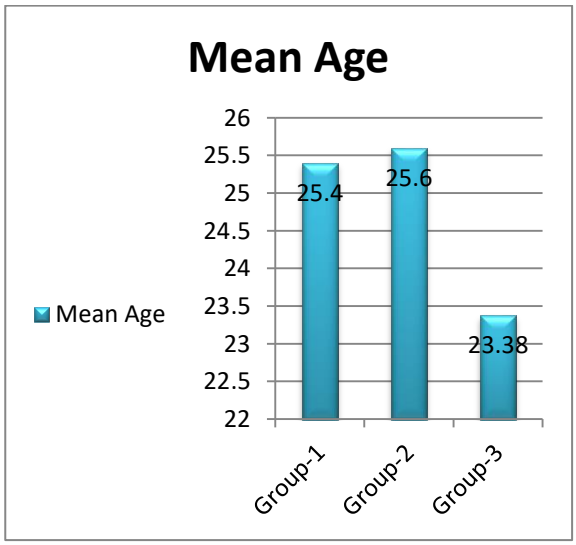
3. Result :

24.19±2.65, respectively) differed statistically significant ($p \leq 0.001$). Also, the mean percentage of PCNA in groups 2 and 3 differed in a highly significantly ($p \leq 0.001$). while no significant difference was found between group 1 and 2, as illustrated by (Table 1, Figure 1).

The expression of PCNA in the trophoblastic tissue of the placenta in different groups of study subjects was estimated. The findings indicated the mean percentage of PCNA positive trophoblastic tissue in the placenta between groups 1 and 3(11.04±2.03 and

Table -1: Body mass index (BMI), PCNA expression, gestational age, and age distribution across the study groups:

Variables Mean± SD	**Group-1 No.=25**	**Group-2 No.=15**	**Group-3 No.=21**	***P value***
Age	25.4±4.8	25.6±3.6	23.38± 3.5	*P > 0.5*
Gestational age	8.4±0.7	8.9±0.9	39.2±1.1	
BMI	23.6±2.4	24.9±2.2	28.3±2.1	*P<0.05*
PCNA	11.04±2.03	15.3±2.7	24.19±2.65	Group1/2 *P* >0.5 Group 1/3 *P* ≤ 0.001 Group 2/3 *P* ≤0.001



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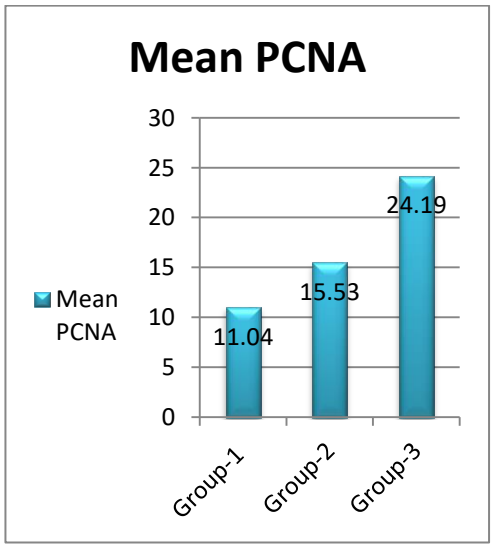

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Figure (1): A: Age distribution of the study groups

B: Expression of PCNA in DSCs by IHC among the studies groups.

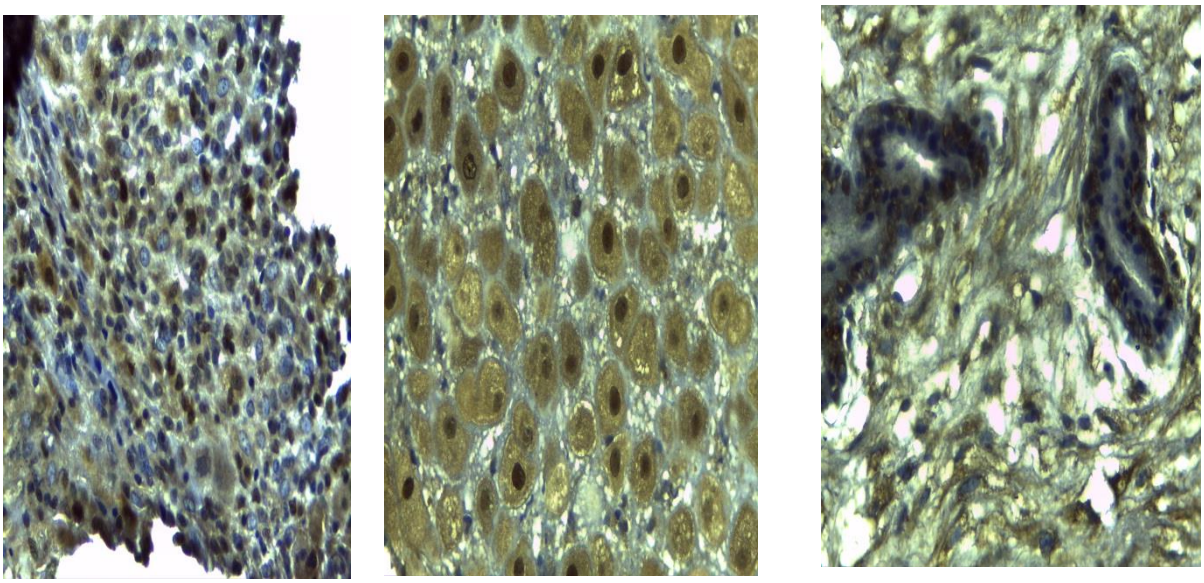

Figure (2): Immunohistochemical staining for PCNA in the DSCs

control group, whereas the level of TNF-α was significantly higher. These data raise the possibility of a correlation that may exist between defective decidual cell proliferation, diverse cytokine profiles, and early miscarriage.

Table (2) shows the comparison of PCNA expression in DSC and changes in serum cytokine (IL-10, TNF-α) levels among the three groups. The percentages of PCNA-positive DSCs and the IL-10 level in both the RM and Non-RM groups were significantly lower than that in the

Table (2): PCNA Expression and Cytokine levels (IL-10, TNF-α) in study groups

Parameter	**Group-1 No.=25**	**Non-Recurrent Miscarriage(N=15)**	**Control (Term pregnancy) N=21**	***p-value***
PCNA positive-DSCs	11.04±2.03	15.3±2.7	24.19±2.65	≤0.001
IL-10 (pg/ml)	38.3±4.7	42.3±3.9	67.3±5.3	≤0.001
TNF-α(pg/ml)	74±6.2	71.3±7.5	46.3±5.1	≤0.001

4. Discussion :

findings are consistent with the present study and reinforce the concept that low proliferation activity is a recurrent and critical feature of early pregnancy loss [17-18].

Immunohistochemical analysis revealed that PCNA expression in normal placentas, as well as in recurrent miscarriage (RM) and non-RM cases, was primarily localized to the cytotrophoblast, with minimal or no staining observed in the syncytiotrophoblast. This distribution supports previous findings indicating that the cytotrophoblast is the main site of trophoblastic proliferation [19–22]. Studies by Ostrzega *et al.* [23], Ozbilim *et al.* [24], and Kale *et al*. [25] examining hydatidiform moles reported significant differences in the PCNA index between partial moles (PM) and complete moles (CM). Additionally, Ozbilim *et al.* [24] and Molykutty *et al*. [26] suggested that PCNA may serve as a predictive marker for persistent trophoblastic disease (PTD). However, such variations in findings may be partly attributed to inherent limitations of PCNA as a marker [27], as well as methodological differences in immunostaining assessment across studies.

The success of early placentation requires a series of coordinated maternal and placental events. Disruption in specific elements of these events has been implicated as early signatures of placental insufficiency leading to miscarriage or fetal growth restriction. The objectives of this study were to evaluate the associations between proliferating cell nuclear antigen (PCNA) expression during first-trimester recurrent miscarriages and some of the main cytokines, such as IL-10 and TNF-α, and to find out any significant relationships that require consideration in screening strategies.

Proliferating Cell Nuclear Antigen (PCNA) is primarily recognized as an accessory protein for DNA polymerase; however, recent studies have revealed its broader involvement in key cellular processes, including cell cycle regulation, DNA repair, and DNA replication. [16].

In this study, PCNA expression was found to be significantly higher in feto-placental tissue from term pregnancy women (24.19±2.65) than that of RM women (11.04±2.03) and non- RM women (15.53±1.3). As a result of these findings, we can conclude that the trophoblasts are actively growing during pregnancy. These

observation is consistent with previous evidence where high TNF-α and low IL-10 levels were associated with early pregnancy loss[29]. A switch to inflammatory conditions may lead to impaired trophoblast invasion and decidual homeostasis, resulting in pregnancy loss [30].

Notably, PCNA and cytokine were not significantly different between recurrent and non-recurrent miscarriage groups, indicating that a similar pathophysiology mechanism is associated with recurrence or not. This finding is consistent with previous reports that immunological and cell abnormalities are found in a variety of miscarriage causes independent of recurrence [31].

Interestingly, cyclosporine A (CsA) stimulates human cytotrophoblast cell migration and PCNA production through NF-κB signaling pathways controlled by MAPK3/1.A previous study indicated that CsA could be used to treat complications of pregnancy caused by deficient trophoblast function due to its importance to placentation and normal pregnancy [28].

Cytokine analysis revealed a notable imbalance in immune regulation. IL-10, as an anti-inflammatory cytokine playing a key role in inducing maternal immune tolerance, was significantly lower in miscarriage groups, TNF-α, a pro-inflammatory cytokine related to trophoblast apoptosis and poor placental growth was significantly higher. This

5. Conclusion:

decidual stromal cells, which may adversely affect receptivity. These results provide supporting evidence for the hypothesis that a dysregulated immune response characterized by an imbalance in central regulatory cytokines and deficient cell proliferation is associated with pregnancy loss. PCNA, IL-10 and TNF-α could be potential biomarkers to assess endometrial function and the risk of miscarriage in early gestation.

The present study demonstrates an inverse correlation between decreased expression of PCNA and the expression level of inflammatory cytokines in first-trimester miscarriage women. The two groups of women with recurrent and non-recurrent miscarriage had decreased IL-10 and increased TNF-α compared to normal pregnancy indicating pro-inflammatory changes in the immune system. Concomitant reduction of PCNA indicates dysfunctional proliferation of

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AI declaration: not applicable

Ethics approval and consent to participate:

This study was approved by the Ethical Committee of the College of Medicine, University of Basrah. Informed consent was obtained in accordance with institutional guidelines.

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