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Decreased β-catenin Expression in First Trimester Villi and Decidua of Patients with Recurrent Spontaneous Miscarriage

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ABSTRACT

Accumulating evidence suggests that Wnt/β-catenin pathways play an important role in the physiological processes of endometrial development and differentiation, preimplantation embryo development, and blastocyst implantation. The objective of the current study was to signify the association between Wnt/β-catenin signalling pathway and recurrent spontaneous miscarriage and examine the expression of β-catenin first-trimester villi and decidua in women with spontaneous miscarriage. A comparative cross-sectional study was conducted in Saladin General Hospital from the 1st of January to the 1st of October 2021. A convenient sample of 80 pregnant women was enrolled in the current study and consisted of group A (40 pregnant women who were presented with first or second miscarriage) and group B (40 pregnant women who were presented with recurrent spontaneous miscarriage). There was a significant association between decreased β-catenin and recurrent miscarriage as 77.5% of the women who presented with recurrent spontaneous miscarriage had decreased levels of β-catenin, while only 32.5% of those with first or second miscarriage had decreased levels of β-catenin. The results of the current study implied that β-catenin is a valuable biomarker for unexplained recurrent spontaneous miscarriage. The women who presented with recurrent spontaneous miscarriage had a decreased expression of β-catenin .

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INTRODUCTION

According to The World Health Organization, miscarriage is defined as the expulsion or extraction of a fetus (embryo) weighing less than 500 gm or before 24 weeks gestation[1, 2], it could be early pregnancy loss when happens before 12 weeks gestation or late pregnancy loss when it happened between 12 and 24 weeks gestation[2]. According to the guidelines of the European Society for Human Reproduction and Embryology and the Royal College of Obstetricians and Gynecologists, recurrent spontaneous miscarriage is often characterized by a sequence of three or more consecutive miscarriages. However, according to the American Society for Reproductive Medicine, it is defined as experiencing two or more successive miscarriages[3].

Certainly, recurrent spontaneous miscarriage is a significant concern in early pregnancy. It is among the more common complications experienced during this stage of pregnancy[4]. Approximately 2-5% of couples in their reproductive years are affected by recurrent spontaneous miscarriage, making it a notable challenge for a significant portion of those trying to conceive [5]. Among clinically diagnosed pregnancies, the rate of pregnancy loss typically falls between 8% to 15%. Most miscarriages, approximately 80%, occur within the first 12 weeks of gestation. After this period, the rates of miscarriage decrease notably as the pregnancy progresses[6, 7]. The World Health Organization reports that around 500,000 women die annually due to pregnancy-related causes, with 98% of these tragic cases happening in developing countries. Among these deaths, approximately 15% are linked to complications arising from miscarriage[7], which brings immense psychological and emotional trauma to women and can also lead to psychiatric distress. Additionally, it can result in economic burdens for families[5]

Recurrent spontaneous miscarriage is a complex condition influenced by genetic, anatomical, autoimmune, endocrine, thrombotic, lifestyle, and maternal infection. Yet, in as many as 50% of cases, the specific cause remains unidentified[6].

The diagnostic assessment should involve examining the genetic makeup of both parents, evaluating the anatomy of the uterus, antiphospholipid antibody syndrome, and certain clotting disorders. For some women, it might also be necessary to assess insulin resistance, ovarian function, antithyroid antibodies, and potential prolactin abnormalities[8]. The prognosis for an individual depends on the reasons behind the pregnancy losses and the number of previous miscarriages[9]. A possible mechanism of the association between prolactin and miscarriage is that high levels of prolactin affect the function of the ovaries, resulting in a luteal phase defect and miscarriage[10]. Insulin resistance is claimed to be a potential cause of recurrent miscarriage in patients with polycystic ovary syndrome and has been linked to metabolic and endocrine abnormalities but the exact mechanism of how insulin resistance leads to recurrent miscarriage in women with normal ovary is unknown[11].

β-Catenin, a highly versatile and evolutionarily preserved molecule, plays a vital role in numerous developmental progression and homeostatic processes. It serves as an essential part of adherens junctions based on cadherins and acts as the primary nuclear factor in canonical WNT signalling. When there is an imbalance in β-catenin's structural or signalling functions, it can lead to irregular growth patterns associated with cancer and metastasis[12].

The lining of the uterus, endometrium, shows specific activity in β-catenin. This protein's behaviour is influenced by estrogen and progesterone. Additionally, β-catenin plays a crucial role in the growth, specialization, and successful attachment of the embryo to the endometrial tissue[13]. Accumulating evidence suggests that the Wnt/β-catenin pathways are significantly involved in the natural processes that drive the development of the endometrium, preimplantation embryo development, blastocyst implantation, and post-implantation uterine decidualization. Even though, the link between irregular Wnt/β-catenin signaling and recurrent spontaneous miscarriages is not yet fully understood or established[14].

The objective of the current study was to signify the association between the Wnt/β-catenin signalling pathway and recurrent spontaneous miscarriage and examine the expression of β-catenin first-trimester villi and decidua in women with spontaneous miscarriage.

PATIENTS AND METHOD

A comparative cross-sectional study was conducted in Iraq, Saladin Governorate, Saladin General Hospital during the period from 1st of January to 1st of October 2021. The study included a group of 80 pregnant women and was categorized into two groups: Group A (included 40 pregnant women in the first trimester who were presented with first or second spontaneous miscarriage and group B (included 40 pregnant women in the first trimester who were presented with recurrent spontaneous miscarriage (three or more).

Exclusion criteria included women who had uterine anatomical disorders (by ultrasound examination), endocrine disorders, infectious diseases during pregnancy, thrombophilia, or cervical incompetence.

The information was collected by conducting direct interviews, physical examinations, and laboratory tests. A questionnaire was developed based on a review of similar articles to gather the necessary details.

In each slide, three randomly chosen fields were analyzed at both 200x and 400x magnifications. The evaluation involved scoring the percentage of cells with cytoplasmic staining, utilizing a specified scale as follow[15]:

- Grade 0 indicates that less than 5% of the epithelial cells within the specific lesions exhibited cytoplasmic staining.
- Grade 1 indicates that 6-25% of the epithelial cells within the specific lesions exhibited cytoplasmic staining.
- Grade 2 indicates that 26-50% of the epithelial cells within the specific lesions exhibited cytoplasmic staining.
- Grade 3 indicates more than 50% of the epithelial cells within the specific lesions exhibited cytoplasmic staining.

The immunohistochemical staining process followed the procedure outlined by Li *et al.* The primary antibody, anti-β-catenin, was diluted at a ratio of 1:50, and the secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG, was diluted at a ratio of 1:200. Negative controls underwent the same steps, except that normal rabbit IgG replaced the primary antibodies.

To quantify the results, the average density of the positive immunostaining signal was assessed using bright-field microscopy.

Ethical considerations

The research was approved by the Scientific Council of Gynecology and Obstetrics under the Iraqi Board of Medical Specializations. Women were asked to participate voluntarily after an adequate explanation of the aim of the study. All participants were assured of anonymity and confidentiality of information.

Statistical analysis

The continuous variables were depicted as mean values along with their respective standard deviations (SD), while categorical data were represented as numbers and percentages. Mann–Whitney U test and Chi-Square test were used to test the statistical difference. A statistically significant result was determined by a P-value below 0.05, indicating a low probability that the observed outcome was due to chance.

RESULTS

A total of 80 women were enrolled, the mean age and standard deviation were 26.11 (±5.94) years. The study groups exhibited significant differences in both body mass index (BMI) and gestational age at the time of miscarriage (P-value<0.05), There was no significant distinction found between the study groups concerning age., as shown in table 1. In group A, 13 (32.5%) of the participants had negative β-catenin expression including grade 0 expression in 9 (22.5%) and weak expression (grade 1) in 4 (10.0%) of the participants, as shown in table 2.

In group B, 31 (77.5%) of the participants had negative β-catenin expression including grade 0 in 24 (60.0%) and weak expression (grade 1) in 7 (17.5%) of the participants, as shown in table 3.

There was a significant difference between the study groups regarding β-catenin expression (P-value<0.05). Most of those in group A had a positive expression, while most of those in group B had a negative expression, as shown in table 4.

Table 1: Age, body mass index, and gestational age distribution of the study groups

		N	Mean ±SD	P-value
Age (years)	Group A	40	32.57 ±6.00	0.877
	Group B	40	32.10 ±6.04	
BMI (kg/m^2)	Group A	40	23.20 ±1.82	**<0.001***
	Group B	40	26.15 ±2.17	
Gestational Age (weeks)	Group A	40	9.82 ±1.83	**0.029***
	Group B	40	8.92 ±1.59	

Table 2: Expression of β-catenin in the group A

β-catenin expression		**N (%)**
Negative expression N=13 (32.5%)	Grade 0	9 (22.5)
	Weak expression (grade 1)	4 (10.0)
Positive expression N=27 (67.5%)	Moderate expression (grade 2)	8 (20.0)
	Intense expression (grade 3)	19 (47.5)
Total		40 (100.0)

Table 3: Expression of β-catenin in the group B

β-catenin expression		**N (%)**
Negative expression N=31(77.5%)	Grade 0	24 (60.0)
	Weak expression (grade 1)	7 (17.5)
Positive expression (N=9 (22.5%)	Moderate expression (grade 2)	3 (7.5)
	Intense expression (grade 3)	6 (15.0)
Total		40 (100.0)

Table 4: Distribution of β-catenin expression according to the study groups

β-catenin	**Groups**		**Total**	
	Group A N (%)	**Group B N (%)**	**N (%)**	**P-value**
Negative expression	13 (29.5)	31 (70.5)	44 (55.0)	**<0.001**
Positive expression	27 (75.0)	9 (25.0)	36 (45.0)	
Total	40 (100.0)	40 (100.0)	80 (100.0)	

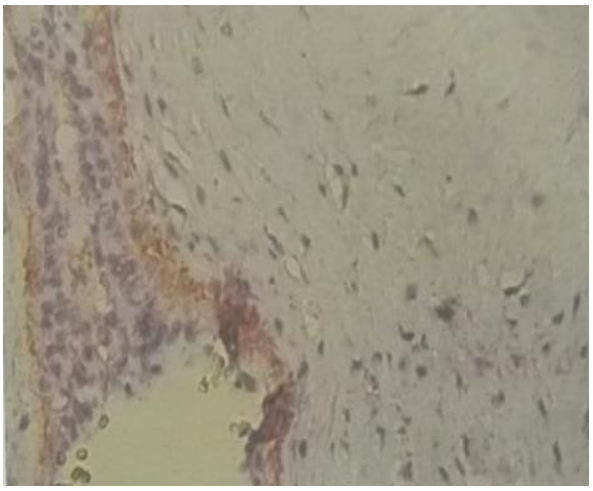

Figure 1: First miscarriage chorionic villi with positive expression of β-catenin (brownish cytoplasm)

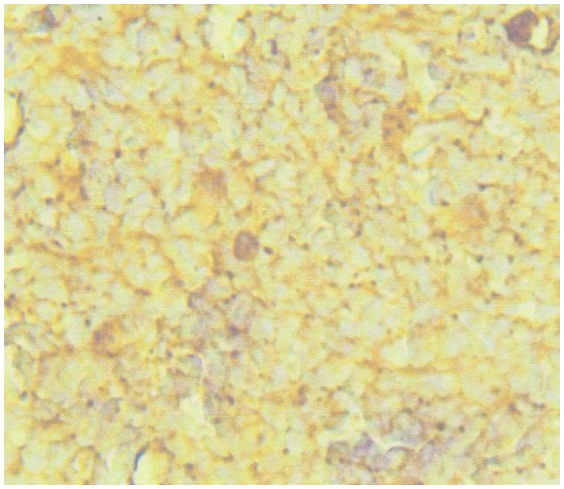

Figure 2: First miscarriage chorionic villi with intense positive β-catenin expression

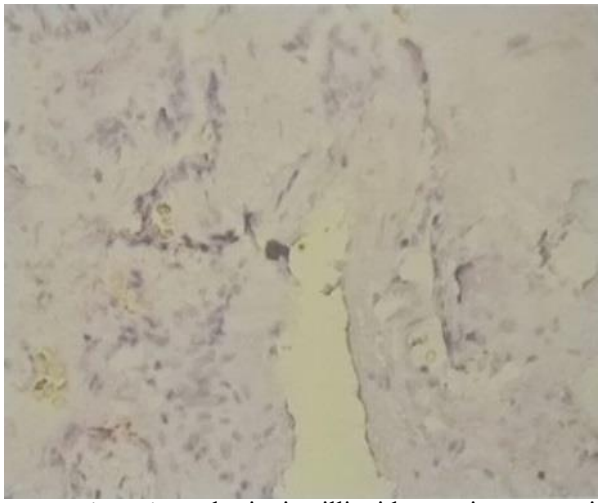

Figure 3: Recurrent miscarriage chorionic villi with negative expression of β-catenin

DISCUSSION

The transformation of proliferative first-trimester cytotrophoblasts into invasive extravillous trophoblasts holds great importance for a successful pregnancy. The canonical Wnt/β-catenin signaling pathway has been considered to play a role in facilitating this differentiation process[16]. This study was the first one to estimate the role and expression of β-catenin in recurrent spontaneous miscarriage in Iraq.

There was no significant association between the study groups regarding age. This disagreed with the results of another study that was done by Dai *et al.* in Cina (2016) which concluded that the increasing maternal age was associated with an increased risk of chromosomal abnormality, fetal abnormalities, and miscarriage[17]. Another study was done by Magnus *et al.* in Norway (2019) which revealed that the chance of miscarriage was related to maternal age[18]. The exclusion criteria of the current study may explain the insignificant difference as most of the age-related factors were excluded.

The mean BMI was significantly higher among women in group B. This agrees with another study that was done in Japan in 2015 and considered obesity as an independent risk factor for further miscarriage in patients with recurrent spontaneous miscarriage and concluded that

Having a BMI greater than 30 kg/m2 is identified as an independent risk factor for subsequent miscarriages. The odds ratio ranges between 1.7 to 3.5 in individuals experiencing early recurrent spontaneous miscarriage, indicating an increased likelihood of further miscarriages in this specific BMI range[19]. A meta-analysis and systematic review that was achieved by Ka Ying et al. to evaluate the links between lifestyle features and recurrent spontaneous miscarriage revealed that BMI > 25 contribute significantly to an increased risk of recurrent spontaneous miscarriage[20]. This may be related to the complication of abnormal body weight.

In this study, a significant link was found between recurrent spontaneous miscarriages and gestational age. Specifically, Group A displayed a higher mean gestational age compared with other groups, indicating a potential association between these variables. In comparison, Bao et al revealed an insignificant difference between women who had recurrent spontaneous miscarriage compared with the control group regarding gestational age at miscarriage[21]. The results of the current study might be related to increased complications with subsequent miscarriages.

The risk of miscarriage may rise to four times after three consecutive prior miscarriages, indicating substantial variability in risk among couples. In another study that was done by Magnus *et al.* in Norway (2019), miscarriage is associated with various past pregnancy complications such as stillbirth, preterm delivery, and gestational diabetes[18].

The main result of the present study was a significant decrease in β-catenin among women with recurrent miscarriage. The results of this study align with a previous study conducted by Shuhong *et al.* in China (2014) which showed the presence of the β-catenin protein in both recurrent spontaneous miscarriage and normal control groups. However, they observed a notable decrease in its expression in the villous and decidual tissues in women who had recurrent spontaneous miscarriages when compared with normal women[16].

CONCLUSIONS

The results of the current study implied that β-catenin is a valuable biomarker for unexplained

recurrent spontaneous miscarriage. The women who presented with recurrent spontaneous miscarriages had a decreased expression of β-catenin compared with women with first or second miscarriages.

RECOMMENDATIONS

Use of β-catenin in the screening and detecting of the causes of recurrent spontaneous miscarriage. Further studies should be carried out in the upcoming period to evaluate the role of β-catenin in the detection and evaluation of recurrent spontaneous miscarriage using a larger sample to predict the impacting factors that could affect the level of β-catenin.

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