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Correlation Between Histidine-Rich Glycoprotein and Thrombospondin-1: Potential Role in Angiogenic Regulation in Idiopathic Recurrent Miscarriage

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ABSTRACT: Background: Recurrent miscarriage (RM) is defined by the European Society of Human Reproduction and Embryology (ESHRE) as the loss of two or more pregnancies, irrespective of their sequence. Several studies have associated angiogenesis with recurrent miscarriage. Moreover, various factors have been identified as participating in the angiogenesis process, including histidine-rich glycoprotein (HRG) and thrombospondin-1 (TSP-1). Objective: The present research set out to investigate the angiogenic function of correlation between TSP-1 and HRG in order to find out its relevance in the pathophysiology of idiopathic recurrent miscarriage.

Material and Methods: A case-control research was performed with 70 women at idiopathic RM and 70 pregnant controls who had at least one live birth. Participants both patients and controls were matched in ages (18-35) years, their husbands ages (20-40) years, and BMI ranged (18.5-29.9) kg/m². Blood samples (10 ml) were obtained from participants. Laboratory testing were employed to exclude probable causes of RM by confirming that they were within normal limits. Stored serum at -80°C were used to examine HRG and TSP-1 concentrations by ELISA technique.

Results: Women with idiopathic recurrent miscarriages had significantly lower levels of histidine rich glycoprotein ($P < 0.001$), whereas higher levels of thrombospondine-1 ($P = 0.00071$) than controls. Furthermore, inverse correlation between these two parameters were noted among patient group. Conclusion: The reduction in HRG, along with the elevation of TSP-1 and the inverse correlation between these biomarkers in women undergoing idiopathic recurrent miscarriage, may result in a dysregulation of angiogenesis, perhaps associated with idiopathic recurrent miscarriages.

INTRODUCTION:

The European Society of Human Reproduction and Embryology (ESHRE) recognized the challenge in establishing a consensus on the definition, suggesting it as the loss of two or more pregnancies. It excludes ectopic and molar pregnancies. Regardless of whether they were consecutive, it was acknowledged that the members did not reach a unanimous consensus on this topic¹. Pregnancy diagnosis presents difficulties, as do the requirements for a conclusive intrauterine pregnancy and its viability. Fetal weight or gestational age might be used to evaluate viability restrictions. Depending on the location, the fetal viability threshold

varies between 20 and 28 weeks of gestation. A miscarriage is defined by the World Health Organization as the evacuation of an embryo weighing less than 500 grams. This usually happens about 22 weeks of gestation². Recurrent miscarriage (RM) can be categorized into two classifications (additionally, other research have delineated RM into three classifications) based on maternal reproductive history, indicating separate pathophysiological mechanisms: (1) primary recurrent miscarriage: Individuals who have a history of recurrent miscarriage (RM) but have never given birth live because every pregnancy has ended in a miscarriage³. (2) Secondary recurrent miscarriage: this condition affects women who have been through one successful pregnancy and have a history of subsequent miscarriages⁴. (3) Tertiary recurrent miscarriage: refers to individuals experiencing three non-consecutive miscarriages⁵. The prevalence of spontaneous abortion varies considerably, estimated between 15% and 40%⁶. In 30-50% of women experiencing recurrent miscarriage, the underlying causes remain unexplained⁷. Various factors lead to recurrent miscarriage, including: genetic factors, anatomical abnormalities, endocrine disorders, immunological factors, thrombophilic disorders, infections, environmental and lifestyle factors⁸.

The process of angiogenesis involves the conversion of existing blood vessels into new ones. It is the master trigger for angiogenesis in the course of development, if not the sole trigger⁹. By enabling the passage of nutrients and waste products, the placenta at the mother-fetal interface provides the fetus with vital metabolic needs for growth¹⁰. The placenta uses angiogenesis to maintain its own circulation and metabolism¹¹. Angiogenesis is classified into two types: The creation of new capillaries from preexisting blood vessels is known as sprouting angiogenesis¹². Instead of outward sprouting, non-sprouting angiogenesis happens through the internal division of an already-existing capillary network¹³.

In 1972, the histidine rich glycoprotein (HRG) was discovered. It is a glycoprotein consists of several domain that is frequently seen in plasma and is primarily produced by the liver¹⁴. It is multidomain structure and ligand-binding capacity allow it to interact with a variety of cells via their surface receptors. As a result, HRG may interact with endothelial, melanoma, ovarian, fibroblast, lymphoma, and myeloid cell lines, as well as EBV-transformed human β -cells, T lymphocytes, murine erythrocytes, and platelets¹⁵. Heparan sulfate, FcR, tropomyosin, and an unknown 50–55 kDa protein on T cells are the four main types of receptors that help HRG attach to cells. The main receptor that facilitates the interaction between HRG and the cell surface is heparan sulfate, which is directly related to the physiological role of HRG¹⁶.

Thrombospondin (TSP) is one of the ligands to which it may bind¹⁷. Five members of the TSP family have been conserved throughout evolution and are consistently expressed in mammalian tissues, TSP-1 and TSP-2 are the primary subjects of recent studies on TSP mechanisms¹⁸. Significant (>400KD) homotrimeric extracellular matrix glycoproteins with intricate domain architectures are TSP-1 and TSP-2, three TSR-1 domains, three EGF-related repeats, a cysteine-rich procollagen-like domain, and a heparin-binding region are all found in the N-terminal region of each monomer. In the C-terminal region, seven calcium-binding repeat domains are followed by a globular domain. Research on the proteolytic digests of TSP-1 revealed that the only regions of the protein with anti-angiogenic characteristics were the procollagen domain and the three TSR-1 domains¹⁹.

Aim of study:

The current study set out to examine the angiogenic function of the relationship between TSP-1 and HRG in order to determine its importance in the pathophysiology of idiopathic recurrent miscarriage.

MATERIAL AND METHODS:

The 140 participants in this case-control research were split into two groups: the first is made up of patients who have experienced first trimester idiopathic recurrent miscarriages, while the second group comprises first trimester pregnant individuals without miscarriage, serving as the control group. The experimental component of this work was executed at the laboratory of the Clinical Biochemistry Department at Al-Nahrain University/ College of Medicine. The sample collection was place from April 10, 2025, to November 19, 2025. Questionnaires were created to gather data from both the control and patient groups.

Patients group

This sample comprised 70 individuals with idiopathic recurrent miscarriage (according to ESHRE definition) occurring during the first trimester of pregnancy (diagnosed using ultrasound examination). Patients aged between (18 to 35) years, with BMI ranged (18.5-29.9) kg/m², and their husband's aged (20 to 40) years. They were admitted to the Emergency Department of Babil Teaching Hospital for Maternity and Children, In addition to the Emergency Department of Imam Al-Sadiq Hospital at Babylon

/ Hilla city. The reasons for their RM was not identified would select according to the diagnosis of gynecologists throughout clinical assessment and laboratory investigation.

Control group

The control group comprised 70 apparently healthy pregnant women who had experienced two or more normal pregnancies outcome and had no previous history of miscarriage, selected from out peoples. This group comprises individuals aged 18 to 35 years, with a BMI ranged from 18.5- 29.9 kg/m², and their husband's aged 20 to 40 years. These women are in 1st-trimester of pregnancy, their pregnancies were confirmed by ultrasound examination and biochemical analysis (a positive serum β -hCG test), furthermore fetal cardiac activity was present in all participants of this group. Fetal viability and pregnancy outcomes were followed up until delivery through mobile phone contact .

The selection of the control group took into consideration matching with the case group in terms of the gestational ages that are obtained by measuring the crown-rump length (CRL) using ultrasonography, number of pregnancies (gravidity), conception method, and prior fertility history.

Exclusion criteria

(1)Women whose RM was determined to be because of: Autoimmune; including anti phospholipid syndrome (APS) and thyroid autoimmune disease. Anatomical; including uterine anomalies, presence of fibrous tissue within the uterus. Endocrine; comprising thyroid dysfunction, hyperprolactinemia, sever polycystic ovary syndrome (PCOS).Thrombophilic defects; women with known inherited thrombophilia were excluded. Infective; as chronic endometritis. Genetic; including fetal chromosomal abnormalities. environmental and lifestyle factors including smokers and obese women ($18.5 < \text{BMI} \leq 30$) Kg/m² are excluded from this study. (2)Ectopic and molar pregnancy by ultrasound. (3)In vitro fertilization (IVF) conception.(4)Women have any medication/drugs including aspirin and heparin. (5) Chronic systemic disease including uncontrolled diabetes (tested HbA_{1c}), chronic hypertension, renal disease (tested urea and creatinine), liver disease (tested liver enzymes ALT and AST). (6)Current vaginal bleeding. (7)Malignancy or history of cancer treatment. (8)History of prior cesarean section. (9)Women whose husbands heavy smokers (≥ 25 cigarettes per day) or alcohol consumers.

Collection of blood samples

Venous blood samples were obtained from both control subjects and patients using a disposable syringe while they were seated or in a semi-reclining position. Ten milliliters of blood were obtained from each patient via venipuncture; 2 milliliters were placed in a sodium citrate tube, mixed gently, and utilized for coagulation test (PT, PTT, fibrinogen), another 2 ml were put into EDTA tube, mixed and utilized for HbA_{1c} analysis, the remaining 6 ml of blood was incrementally transferred into disposable tubes containing isolating gel. Meanwhile, the remaining blood in gel-containing tubes was allowed to coagulate at room temperature for 30 minutes, subsequently centrifuged at 4000 rpm for ten minutes, and then divided into two portions using Eppendorf tubes; the first Eppendorf tubes was used for female sex hormone (FSH, LH, progesterone, estradiol, testosterone, prolactin), thyroid functions (TSH, T₃, T₄), liver functions (ALT, AST), and renal functions (Urea, Creatinine), immunology (APA-IgM, ACL-IgM, Lupus anticoagulant) investigations to verify that the results are within normal reference ranges to exclude etiologies of recurrent pregnancy loss utilizing fully automated process . Second Eppendorf; divided into small aliquots and stored at -80°C until analysis for the quantification of HRG and TSP-1 via enzyme-linked immuno-sorbent assay (ELISA).

Determination of Human Histidine Rich Glycoprotein (HRG) in Serum

The calculation was performed using the ELISA kit as per the specifications of the Manufacture Company/ BT LAB located in China.

Determination of Human Thrombospondin-1 (TSP-1) in Serum

The calculation was performed using the ELISA kit as per the specifications of the Manufacturing Company/ BT LAB /China.

Statistical analysis:

The data was analyzed utilizing the Software Package for Social Science (SPSS Statistics 31.0.2). The means $\pm$ SD were compared using a t-test in SPSS Statistics. Correlations was utilized to examine potential correlations among these biomarkers. The

diagnostic effectiveness of HRG and TSP-1 in distinguishing between patients with idiopathic RM and controls was evaluated using the receiver operating characteristic curve.

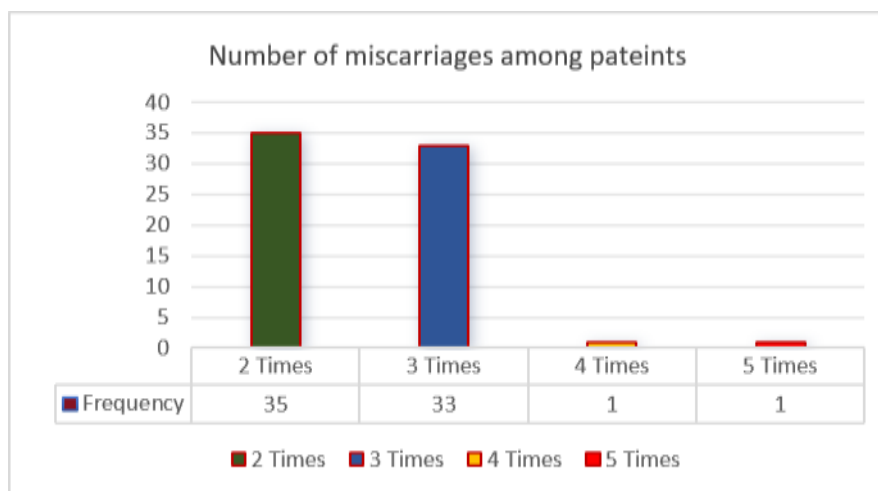
Results:

The mean difference of demographic characteristics between patients and control groups are statistically non-significant ($p > 0.05$). these demonstrated in Table (1):

Table (1): Mean $\pm$ Standard Deviation (SD) of Ages, Husband's Ages, BMI, Pregnancy Duration, and Gravidity in Patients and Control Groups

Parameter	Groups	No.	Mean $\pm$ Std. Deviation	P. value
Ages (years)	Patients	70	28.11 $\pm$ 5.020	0.849
	Control	70	28.27 $\pm$ 4.693	
Husband's Ages (years)	Patients	70	32.16 $\pm$ 5.555	0.851
	Control	70	32.33 $\pm$ 5.205	
BMI (Kg/m ²)	Patients	70	24.714 $\pm$ 2.166	0.106
	Control	70	25.379 $\pm$ 2.541	
Gestational Ages (weeks)	Patients	70	8.1143 $\pm$ 2.836	0.855
	Control	70	8.0286 $\pm$ 2.718	
Gravidity	Patients	70	3.613 $\pm$ 1.351	0.388
	Control	70	3.436 $\pm$ 1.172	

The frequency distribution of miscarriages among the study participants is demonstrated in the figure (1).



Figure(1): Frequency of miscarriages in patients

When compared to the control group, the levels of HRG in patients group were significantly lower ($P < 0.001$). according to the table (2).

Table (2) Mean $\pm$ Standard Deviation (SD) of HRG concentration in patients and control groups

Parameter	Groups	No.	Mean $\pm$ Std. Deviation	P. value
HRG (ng/ml)	Controls	70	183.36331 $\pm$ 84.375347	<0.001
	Patients	70	109.93319 $\pm$ 41.195798	

The levels of Thrombospondin-1 in patients were significantly lower ($P = 0.00071$), when compared to control. These data presented in the table (3):

Table (3) Mean $\pm$ standard deviation (SD) of TSP-1 concentration in patients and control groups

Parameter	Groups	No.	Mean $\pm$ Std. Deviation	P. value
TSP-1 (ng/dl)	control	70	88.33429 $\pm$ 17.486352	0.00071
	patients	70	122.38127 $\pm$ 59.660960	

Correlation between HRG and TSP-1

The study's results are displayed in tables (4), which indicate the correlation between these parameters.

Table (4): Biochemical parameters correlations

		HRG	TSP-1
HRG	Pearson Correlation	1	-.352-**
	P. value		.003
TSP-1	Pearson Correlation	-.352-**	1
	P. value	.003	

According to the findings, there is a negative association ($p=0.003$; $r=-.352-$) between HRG and TSP-1 in the patients. This is observed in figure (2):

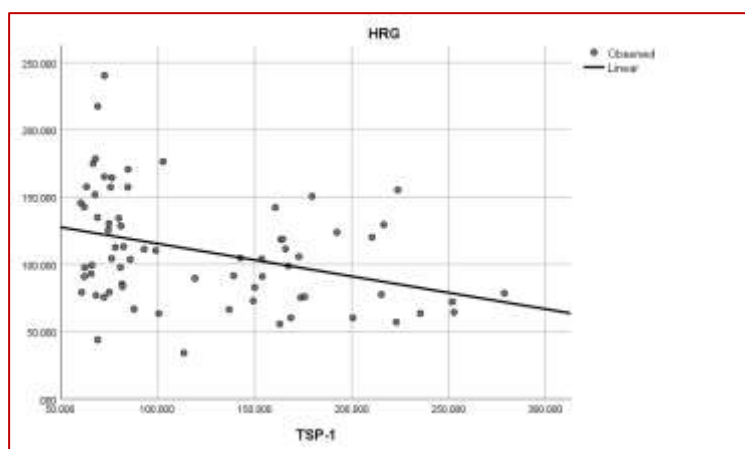


Figure (2): Correlation between HRG and TSP-1

As illustrated in table (5), TSP-1 and HRG levels were shown to have a statistically significant inverse relationship ($b1 = -0.243$, $P = 0.003$). Nevertheless, The low coefficient of determination ($R^2 = 0.124$) indicates that TSP-1 accounts for just a small fraction of the variability in HRG, suggesting that additional biological or clinical variables may significantly influence HRG levels.

Table (5): Linear Regression Analysis Between TSP-1 and HRG

The dependent variable: HRG							
Equation	Model Summary					Parameter Estimates	
	R Square	F	df1	df2	Sig.	Constant	b1
Linear	.124	9.626	1	68	.003	139.691	-.243-
The independent variable: TSP-1.							

ROC Curve for HRG

The HRG ROC curve displayed in Figure (3):

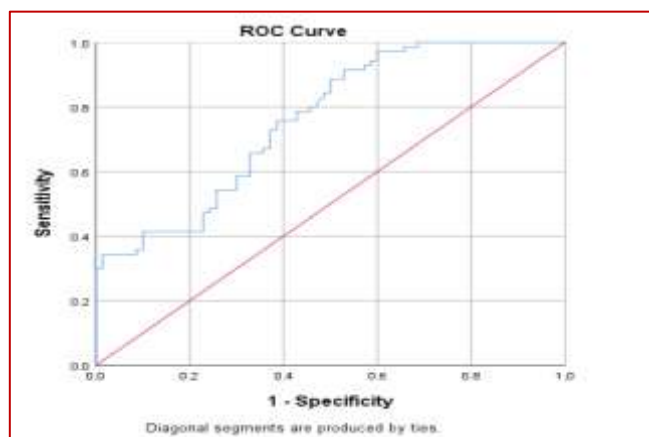


Figure (3): ROC Curve for HRG

The area under curve was (75.9%), cut off value was (135.706), while sensitivity and specificity values were (75.7%) (61.4%) respectively. This is seen in the following table (6):

Table (6) The Area Under Curve and Cut Off for HRG

Area	Cutoff value	Sensitivity	Specificity	P. value	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
75.9%	135.706	75.7%	61.4%	<0.001	0.681	0.836

With an AUC of 0.759 (95% CI: 0.681–0.836, $P < 0.001$), ROC curve analysis showed good diagnostic performance. The marker's sensitivity was 75.7% and specificity was 61.4% at the ideal cutoff value of 135.706, suggesting an acceptable capacity to distinguish between cases and controls. The biomarker had just moderate discriminatory capacity, indicating restricted independent diagnostic utility. Despite being statistically significant, the poor specificity suggests a substantial incidence of false-positive outcomes. This marker may be more beneficial as a component of a composite panel rather than as a standalone diagnostic test.

ROC Curve for TSP-1

The TSP-1 ROC curve illustrated in figure (4):

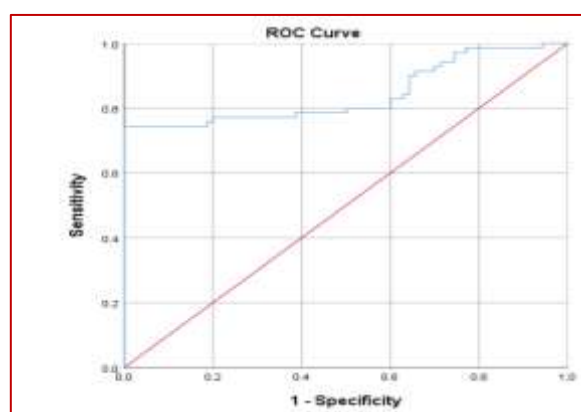


Figure (4): ROC curve for TSP-1

The area under curve (AUC) was (84.4%), cut off value was (100.488), while sensitivity and specificity values were (77.1%) (80%) respectively. As observed in the table below (7):

Table (7): The area under curve and cut off for TSP-1

Area	Cutoff Value	Sensitivity	Specificity	P. value	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
84.4%	100.48875	77.1%	80%	<0.001	0.774	0.914

With an AUC of 0.84 (95% CI: 0.77–0.91, $P < 0.001$), receiver operating characteristic curve analysis showed satisfactory diagnostic performance. Sensitivity was 77% and specificity was 80% at the ideal cutoff value of 100.49, demonstrating the marker's high capacity to distinguish between cases and controls. The results indicate that the biomarker examined possesses significant discriminative capacity in distinguishing between patients and controls. The marker may possess diagnostic relevance, as suggested by the relatively high AUC. Its limited sensitivity and specificity indicate that it should be utilized with a panel of biomarkers rather than as an independent diagnostic instrument.

DISCUSSION:

In order to provide strong statistical results, it is imperative that patients and control groups match in ages, match in their husbands ages, BMI, pregnant duration and gravidity, to reduce variability in results that may occur from differences in these features. The study's participants ranged in age from 18 to 35 for women and from 20 to 40 for husbands. In line with earlier research, which found that women aged (35.0–39.9) years and ≥ 40.0 years had a significantly elevated risk of miscarriage, while husbands aged ≥ 40.0 years demonstrated the greatest risk of miscarriage, advanced parental ages were avoided because they were thought to be a risk-factor for recurrent miscarriage²⁰. According to research by Julia Tomkiewicz and Dorota Darmochwal-Kolarz, Women in the following age groups have an increased risk of miscarriage: (12–19) years old (13%), (20–24) years old (11%), (25–29) years old (12%), (30–34) years old (15%), (35–39) years old (25%), (40–44) years old (51%), and 45 and up (93%). Furthermore, a higher incidence of miscarriage is associated with older fathers. As maternal age increases, the prognosis worsens²¹. In this investigation, In order to prevent increased risk for RM, participants with underweight BMI and obesity were removed from the study. This is consistent with other studies, including one by H. Lashen and K. Fear *et al*, which shown that obesity is linked to an elevated risk of first trimester and recurrent miscarriage²². Although the results of another research by Marcelo B. Cavalcante and Manoel Sarno *et al*, indicated that overweight women were not at risk for RM, it appeared that obese women who had previously experienced recurrent pregnancy loss (RPL) were more likely to experience additional pregnancy losses²³.

According to this study, patients with idiopathic RM had lower levels of histidine rich glycoprotein compared to the group under control. Several research have reached similar conclusions, including one by Xiaohe Sun, Jiamin Jin, *et al*, which found that patients with spontaneous RM had lower HRG protein concentrations than patients in the control group²⁴. Moreover, according to a study by Karin E. Lindgren and Julius Hreinsson *et al*, adequate implantation and placentation are necessary for a normal pregnancy. These processes rely on balanced angiogenesis as well as appropriate regulation of coagulation and inflammation. Histidine-rich glycoprotein (HRG) is an abundant and important plasma protein for angiogenesis, coagulation, immunological response regulation, and fibrinolysis²⁵. Agreeing to a different research by Marie Bolin and Peter Åkerud *et al*, all pregnant women had reduced levels of HRG, but those who eventually developed preeclampsia had considerably lower levels at gestational weeks 10, 25, and 28 compared to normal pregnant women²⁶. Previous research indicates a relationship between the risk of pre-eclampsia in pregnant women and the existence of HRG in the placenta, which may be the primary cause of the implantation and placentation failure²⁷. Although there is still little research on HRG in RM, a number of studies point to its possible role in angiogenic properties for females during pregnancy, in one of these research, Sarah Nordqvist and Karin Kårehed *et al*, found HRG in the placenta, follicular fluid, female reproductive system, and embryos. Additionally, blastocysts were found to express HRG. Therefore, HRG's angiogenic qualities may have an impact on fertility²⁸. Another study on the function of HRG in embryos found that human embryos begin producing HRG at the 4-cell stage and continue to do so until

the blastocyst stage. Nearly every trophectoderm cell (TE) and inner cell mass (ICM) cell has HRG. Additionally, during culture, embryos release HRG into the surrounding medium¹⁴. HRG enhances angiogenesis by inhibiting thrombospondins 1 and 2 and interfering with thrombospondin's binding to its receptor CD₃₆, which is expressed via the second N-terminal in the endometrium of the human uterine lining²⁹

According to a study by L. Ling-yun and W. U. Xin *et al*, abnormal expression of TSP-1 in villus tissues is linked to defective angiogenesis at the maternal-fetal interface in patients with recurrent miscarriages, it is just one of many studies that have reported that TSP-1 plays an important role in placental vascular development and trophoblast invasion³⁰. This finding was in contrast with a study conducted by Jianing Hu and Chuanmei Qin *et al*, they found that endometrial stromal decidualization is impaired by lower levels of thrombospondin-1 (TSP-1), which is substantially linked to spontaneous idiopathic RM, TSP-1 is typically aid in the uterine lining's metamorphosis during pregnancy; its absence may interfere with this process³¹. Yan Jin and Xipeng Wang *et al*, conducted another study to investigate the impact of TSP-1 on decidual macrophages implicated in the vulnerability to idiopathic spontaneous recurrent miscarriage; they found that certain women may experience pregnancy loss due to unusual expression of TSP-1³². T. Özçakır and M.A. Turan *et al*, conducted another study regarding the potential role of molecules like the role of TSP-1 in angiogenesis in the causes of missed abortions. They discovered that while the endometrial glands had a little increase, the number of blood vessels in the chorionic and decidual areas of the missed abortion cases was much lower than that of a typical pregnancy. They also observed that TSPs immunoreactivities of syncytiotrophoblasts were significantly stronger than those of the missed abortion group³³. According to the current findings, increase TSP-1 levels have been linked to poor angiogenic balance and pregnancy complications, which may be association with recurrent miscarriages.

This finding demonstrated a correlation between lower HRG and higher TSP-1 levels in the patient group. The multifunctional nature of HRG is partially explained by its interaction with a variety of different ligands, including thrombospondins (TSPs) and it is suggested that it acts as an adaptor protein ³⁴. Reduced HRG may enhance the dominance of TSP-1 anti-angiogenic properties, according to a research by Lindgren *et al*, thrombospondins (TSPs) are strong angiogenesis inhibitors that block the response of many growth factors, including as FGF-2 and VEGF, when they bind to their receptor CD₃₆. Two CD₃₆ homology domains (also known as CLESH-1 domains) in the N2 and C-terminal domains of HRG bind to TSP-1 with high affinity, preventing its anti-angiogenic function ¹⁴. The identified inverse connection between HRG and TSP-1 suggests a disrupted angiogenic equilibrium in women experiencing idiopathic RM, decreased HRG levels may attenuate its modulatory influence on TSP-1, thereby augmenting the anti-angiogenic properties of TSP-1 and this may be association with RM, this according with M.C. Krog, E.M. Flachs *et al*; In women with spontaneous RM, angiogenesis-related biomarkers exhibited time-dependent alterations throughout early pregnancy prior to a pregnancy loss, in contrast to those resulting in live delivery³⁵.

CONCLUSION:

The decrease in HRG along with the increase in TSP-1 with a negative relationship between these biomarkers in individuals experiencing idiopathic recurrent miscarriage might lead to an imbalance in angiogenesis regulation, this might be linked to idiopathic recurrent miscarriages.

Ethical and Scientific Approval:

The study protocol was approved by the Research Ethics Committee / Institutional Review Board (IRB) at Al-Nahrain University/ College of Medicine (Ref. No. 214/3/2; Date: 10 March 2025).

Prior to sample collection, all study participants were informed, and their written consent was acquired.

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