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Role of Angiopoietin-1 and 2 as Angiogenic Factors in Women With Abnormal Uterine Bleeding

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ABSTRACT

Human uterus is the principal place where angiogenesis occur every month in physiological setting. Abnormal angiogenesis is associated with abnormal uterine bleeding. Angiogenesis, or vascular growth, is therefore an essential component for growththat occurs in endometrium during every cycle of menses.The Angiopoietin family of growth factors along with VEGF family, mostly VEGF-A which are essential for vascular development are involved in endometrial repair mechanism. Angiopoietin binds to its receptor Tie-2 and induce autophosphorylation of receptor and promotes endothelial cell proliferation and finally vessels formation. The purpose of this study was to evaluatewhether idiopathic excess menstrual bleeding is associated with alteration of circulating angiopoetin-1 as well as angiopoietin-2 in women diagnosed with abnormal uterine bleeding and to correlate with control population. This case-control study was conducted on 120 females aged between 18-52 years with history of bleeding excessively for more than 3 months and 120 controls aged between 19-45. Serum Angiopoietin 1 and 2 were measured by ELISA kit method.

Keywords: *Abnormal uterine bleeding (AUB), Angiopoietin (Ang), Angiogenesis.*

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INTRODUCTION

Abnormal uterine bleeding (AUB) is defined as immoderately heavy, with long duration and frequent uterine bleeding that is not caused by pregnancy related complication or any underlying pelvic or systemic problem [1].It is due to an unknown etiology and therefore referred as diagnosis of exclusion. Among the women with complain of excess menstrual bleeding, 40 to 60% are diagnosed with abnormal uterine bleeding of unknown etiology which is defined as a blood loss of more than 80ml per cycle (normal menstrual loss <80 mL) [2-4]. There are wide range of complication may arise as a result of heavy blood loss, such as iron deficiency anaemia which is primary manifestation of AUB [5-7]. This could affect women's health with respect to medical and social perspective. Emotional, social and decreased quality of life as a result of abnormal uterine bleeding is a challenging area for treatment, along with other existing symptoms [8]. AUB is one of the common reason for iron deficiency in the developed countries and chronic illness in the developing countries [9].AUB is classified into two distinct groups: ovulatory and anovulatory. Ovulatory AUB accounts for about 80% of cases. In ovulatory AUB the menstruation is regular, preceded by ovulation and excessive but of normal duration. It is usually occur in women in their 30s. Anovulatory AUB is more likely to occur at the beginning and end of the reproductive years. The menstrual cycle is irregular and the bleeding is heavy and may be prolonged[10]. Alteration of angiogenic phenomenon are major attributing factor effecting the pathophysiology of menstruation. The variety of these factors found to play a key role in abnormal menstrual bleeding [11]. Angiogenesis is the development of new microvessels from already existed blood vessels and it involves microvascular endothelial cells. Physiological angiogenesis does not occur frequently in adults except during the process

of wound healing, and in the ovary and the endometrium when the women are in their reproductive life[12].Angiopoietins are secreted multimeric growth factors that were first found as ligands for Tie2, but since then have been also found to exert their effects by binding various integrins under specific conditions. Three angiopoietins can be found in humans: angiopoietin 1, 2, and 4. They share a very similar overall structure, but differ in terms of expression pattern, efficiency for Tie2 activation, and signalling outcome. Ang1 and Ang2 are the most studied ligands in the Ang/Tie pathway mediating opposing functions on Tie2 signalling in terms of vessel integrity[13].Ang4 and its mouse orthologue Ang3 are less studied ligands in this pathway and little is known of their contribution to normal and pathological endothelial cell biology. This study was based on the hypothesis that excessive menstrual bleeding changes the physiology of endometrium and aimed to investigate the two important angiogenic biomarkers, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) that plays a critical role in rebuilding of functional layers of endometrium as well as stabilizing the newly synthesised blood vessels during post-menstruation and to find out whether these angiogenic biomarkers work in a different way in abnormal uterine bleeding in those with unknown etiology.

METHODS

Patients and sampling

Ethical clearance from the Institutional Ethical committee (IEC 188/2013) was obtained to conduct this study.The patients and control population were non gravid women of reproductive age visiting the department of OBG, Kasturba hospital, Manipal. They were selected from the same geographical area to avoid any environmental factor and dietary factor that may have an effect on results. One hundred and twenty consecutive patients who underwent endometrial biopsy for AUB were recruited into the study. The control group (n=120) were selected after intensive preliminary investigation among healthy women with regular menstrual cycle with no systemic disease visiting health check-up department, Kasturba Hospital, Manipal. The uterine cavity of patients with AUB appeared normal. Women with menstrual blood loss of more than 80 mlper menstrual cycle selected for this study. Subjects for this study selected among those not, smoked, or had used drugs or hormonal or intrauterine contraception for at least 3 months before biopsy, or had abnormal preoperative values for blood platelets, activated prothrombin, thromboplastin time, international normalized ratio or bleeding time. Women with symptoms of dysmenorrhea and dyspareurenia, and a history of endometriosis, were excluded from the study. Other important factors that could effect menstrual bleeding such as high BMI, diabetes mellitus, hypertension and hypo and hyperthyroidism was ruled out. Those who were pregnant as well as women with other disorder such as fibroids, polyps and tumors were excluded from the study.

Statistical Analysis:

All data were analyzed by SPSS 22. Normally distributed data are expressed as mean±SD and others as Median with IQR (Inter quartile range). Receiver operating characteristic (ROC) curves were used to determine the optimal cut-off values. The optimal cut-off points for each parameter measure were determined by the point ofconvergence of sensitivity and specificity.

RESULT

Mean Age of the patients participated in this study was 39.25 years and mean age for controls was 34.14 years. When serum Angiopoietin-1estimated in patients with AUB as well as control group, there was a negative correlation found with Angiopoietin-2 among the patient group but positive correlation observed in control group. The expression levels of serum angiopoietin-1 was expressed in the form of median with interquartile range. When the serum concentration of Angiopoietin-1 was compared between AUB patients and controls, it is found to be lower in patient's group 17.29 (11.46,30.86) pg/ml, and in healthy controls was 21.42 (8.5,28.41) pg/ml, median with IQ range, data are presented in table 1. A difference in the level of angiopoetin-1 between patient and control group (women with regular menstrual cycle) was not statistically significant ($p<0.581$).The serum angiopoietin-2 measured in the same patients51.64±8.5pg/ml and in controls 22.34±6.54 pg/ml and it is found to be statistically significant $p<0.001$. [Table 1].

The correlation coefficient between Angiopoietin-1 and angiopoietin-2 among the cases showed a negative correlation ($r=-0.117$) which was statistically significant. Angiopoetin-1 secreted by perivascular cells is thought to promote the production of smooth muscle cell mitogens such as platelet-derived growth factor and heparin-binding epidermal growth factor in the endothelium. The angiopoetin-1 contributes to the repair and regeneration of endometrium. This disturbance in the process of endometrial angiogenesis may result in menorrhagia and multiple abnormalities in endometrial microvascular morphology. The strong positive correlation coefficient ($r=0.612$) obtained between angiopoietin-1 and 2 among the control population which was statistically significant ($p<0.001$). [Table

2]. Based on the ROC curves for each of the biomarkers (angiopoietin-1 and angiopoietin-2), the threshold cut-off values with highest sensitivity and specificity were identified and are depicted in table 3. [Figure 1 and 2]. The cut-off value obtained for angiopoietin-1 was 12.1 pg/ml and angiopoietin-2 has 24.7pg/ml. Angiopoietin-1 shows only 33.3% specificity and it not in close proximity of sensitivity. Aniopoietin-2 has sensitivity approximates specificity.[Table 3].

The calculated areas under the ROC curves for angiopoietin-1 and angiopoietin-2 ranged 0.534, 0.943 respectively. The result indicate highest value obtained for area under the curve for angiopoietin-2 and high sensitivity for both analyzed angiogenic biomarkers were representative of sensitivity and specificity of test and probably could have more diagnostic value in patient with AUB.

Table 1: Result of angiopoetin-1 and angiopoietin-2 among the patient and control group.

	Patients (n=120)		Controls (n=120)		P value
Biomarkers	**Range**		**Range**		
Age (years)	18-52	39.25±7.77	19-45	34.14±7	<0.27
Angiopoietin-1 (pg/ml) median IQR	1.63-51.43	17.29 (11.46,30.86)	1.23-57.71	21.42 (8.5, 28.41)	<0.581
Angiopoietin-2 (pg/ml) Mean±SD	29-69.3	51.64±8.5	7.43-36.76	22.34±6.54	<0.001

Table 2: The correlation coefficient between angiopoietin-1 with angiopoietin-2 in AUB patients and control group.

Group			**Angiopoietin-2**
AUB patients	**Angiopoietin-1**	**r**	**- 0.177**
		p	**0.05**
		n	**120**
Healthy controls	**Angiopoietin-1**	**r**	**0.612**
		P	**0.001**
		n	**120**

r= correlation coefficient p=statistical significance n= number of subjects

Table 2 represents a correlation coefficient values between angiopoetin-1 and angiopoietin-2 in both patient and control group. A negative correlation is observed between angiopoetin-1 and angiopoietin-2 among the patients but among control group a strong positive correlation observed between these two variable.

Table 3: Sensitivity and Specificity of serum angiopoietin-1 and angiopoietin-2 in patients with AUB.

Variables	**Cut-off**	**Sensitivity**	**Specificity**	**Area under the curve**
Angiopoietin-1 (pg/ml)	**12.1**	**70%**	**33.3%**	**0.534**
Angiopoietin-2 (pg/ml)	**24.7**	**87.7%**	**77.8%**	**0.943**

The area under the ROC curves for angiopoietin-1 and angiopoietin-2 ranged 0.534 and 0.943 respectively. Cut off point for angiopoetin-1 was 12.1 pg/ml that is lower than the value obtained for both case and control group. The high specificity and sensitivity for angiopoietin-2 is representative of clinical significance of this biomarker.

Figure 1: the empirical ROC curve depicted for Angiopoietin-1

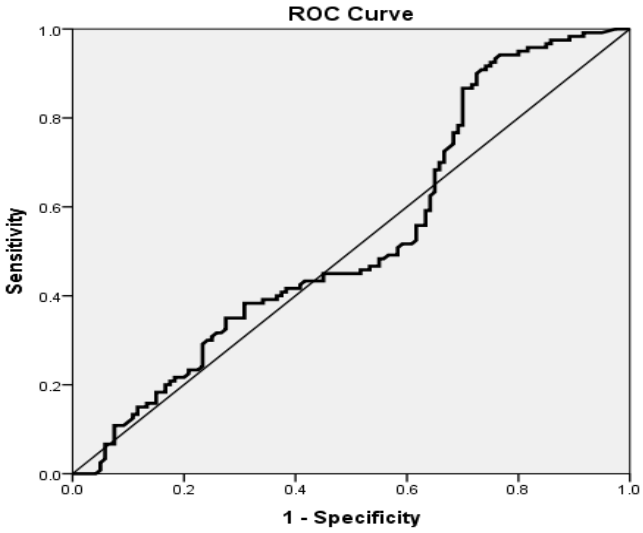
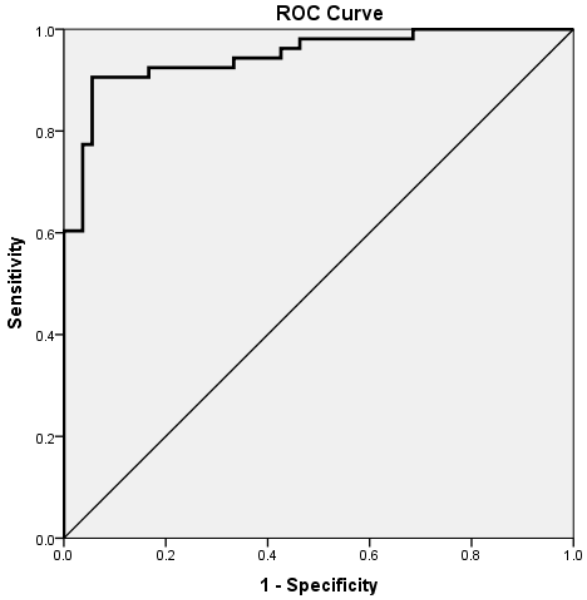


Figure 2: The empirical ROC curve depicted for Angiopoietin-2



DISCUSSION

In this study we found a weak negative correlation between aniopoietin-1 with angiopoietin-2 among the patient group, and serum concentration of angiopoietin-1 was lower than control group. The analysis of angiopoietin-2 revealed higher concentration among the patients as compared with control group. The result of this study clearly prove the hypothesis thatangiopoietin-2 opposes the action of angiopoietin-1 in higher concentration and as result of this abnormal phenomenon the physiological action of angiopoietin-1 in newly formed blood vessels prevented and consequently leads to formation of fragile blood vessel and finally bring about the frequent endometrial break down that is the manifestation of abnormal uterine bleeding. Angiogenesis is vital for development and differentiation of human endometrium that is necessary for implantation, as well as for maintenance of pregnancy[14]. There are varies factor that causes the occurrence of abnormal angiogenesis that is one of the main manifestation of abnormal uterine bleeding. There are numerous factors involved in angiogenesis, Local hypoxia, which follows withdrawal of progesterone has a crucial role in postmenstrual endometrial repair. The hypoxia inducible factor-1 (HIF-1) seems to co-ordinate tissue responses. This factor mediates its action through two subunits, HIF-1 α and HIF-1β. Activation of HIF-1 which take place in absence of oxygen, enhances transcriptional activity of genes with hypoxic response elements, in particular, factors which mediate endometrial remodelling and angiogenesis [15-17]. Therefore, menstruation as an inflammatory process may have a direct impact on post-menstrual tissue repair. Increases in vasodilatation and lack of vasoconstriction associated with AUB condition, may limit or prevent the perimenstrual hypoxic event in the regrowing zone of endometrium. As a result, the endometrial repair process may be delayed. Non hypoxic conditions cause inactivation of HIF-1, which may cause a decrease in the transcription of angiogenic repair factor, and subsequently may prolong the menstrual bleeding. VEGF is one of the genes targeted by HIF-1. Some authors found a decrease in the VEGF mRNA levels in the menstrual effluent and in endometrium biopsied in women with AUB [18]. This low level of VEGF-A would detrimentally affect migration and differentiation of vascular smooth muscles and contributes to defective initiation of coagulation. As a result, delay in repair the damaged vessels and increase in menstrual bleeding may result. Up regulation of the expression of COX enzymes and prostaglandin receptors enhance the production of angiogenic factors and down-regulate the expression of anti-angiogenic factors. Many angiogenic factors are up regulated by COX enzymes in the endometrial epithelial cells such as bFGF, VEGF, and angiopoietin. The process that angiopoetin-1 (Ang-1) stimulate blood vessel formation is via Tie-2 receptor, while angiopoetin-2 (Ang-2) is antagonist of Ang-1 and involve in vessel destabilization and initiates neovascularization in the presence of vascular endothelial growth factor. To validate the hypothesis of this study that AUB may arise as a result of inappropriate signalling cascade of vasculature, the concentration of angiopoetin-1 along with other angiogenic factors in women with AUB was studied but no statistical significant difference was observed between case and control with respect to angiopoetin-1 and slightly higher concentration was observed in control population. The angiopoietin-2 concentration found to be higher in AUB patients when compared to controls. During menstruation, shedding of the functional layer of the endometrium occurs followed by intense vasoconstriction of the

remaining basal arteriolar fragments, which prevents excessive blood loss until the damaged surrounding tissues and blood vessels are repaired and regenerated. It is clear from this study and evident from previous studies that abnormal angiogenesis could be one of the underlying causes of AUB and more studies and further investigation may improve the treatment outcome.

CONCLUSION

In analysis of serum sample of women with AUB we observed decrease in angiopoetin-1 and over expression of angiopoietin-2 concentration which may contribute to disturbed endometrial vascular remodelling in this condition since the other studies have been reported endothelial cell proliferation is significantly greater in AUB patients. This implies a continual process of vascular remodelling in endometrium of AUB patients throughout the menstrual cycle.

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DISCLOSURE SUMMARY: The authors have no conflicts of interest to declare.

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