

Serum Copper Levels in Women with Dysfunctional Uterine Bleeding

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ABSTRACT

Aim: Dysfunctional uterine bleeding (DUB) is believed to be regulated by angiogenic growth factors under the influence of ovarian steroids primarily by vascular endothelial growth factor (vegf) which can be induced by physiologically relevant concentrations of copper. Hence we hypothesized that elevated serum copper levels could be associated with DUB.

Methods: Blood samples were collected from thirty two female patients suffering with DUB as well as age matched control females. Serum copper levels were estimated using 3, 5-di bromo pyridazole sulphanic acid as coloring agent.

Results and Conclusion: Levels of serum copper in DUB patients demonstrated a 1.7 fold increase and was significantly higher ($p < 0.001$) when compared to controls. This study provides an evidence that higher levels of serum copper is present in endometrial DUB.

Key words: Angiogenesis, DUB, serum copper.

INTRODUCTION

The menorrhagia, which is excessively heavy menstrual bleeding in the absence of a well defined pelvic pathology is termed as Dysfunctional Uterine Bleeding (DUB). It may be due to tumors of the ovary, uterus, cervix, introduction of copper containing Intra uterine Device (IUD) or ovulatory dysfunction due to failure of normal progression in cyclic hormonal stimulation of endometrium. ⁽¹⁾ DUB occurs from the endometrial capillaries and smaller vessels and is a common side effect of progestin-only contraceptives and a wide range of other hormonal conditions. ^{(2),(3)} Understanding of the cellular mechanism that lead to DUB remain elusive because of both the wide range of hormonal conditions under which it can occur and the high degree of variability between different women in terms of their endometrial response to exogenous hormones and their susceptibility to DUB. Thus, the link between hormones and DUB is not direct ^{(4),(5)}.

Endometrial angiogenesis is thought to be under the control of angiogenic growth factors, which in turn is controlled by ovarian steroids mainly by Vascular Endothelial Growth Factor (VEGF) and its receptors VEGF-1 and VEGF-2. ⁽⁶⁾ The mechanism how VEGF regulates endometrial angiogenesis is yet to be established, probably there may be large shifts in

endometrial expression of VEGF that may lead to DUB ^{(7),(8)}. There are reports of elevated VEGF in the endometrium of (Levonorgestrel, synthetic progestogen) Norplant® users which is in favor of the claim that alteration in VEGF expression can cause DUB ^{(9),(10)}.

A specific amount of copper appears to be vital for angiogenesis to occur. ^{(11),(12),(13)} Copper sulfate induces VEGF expression at a concentrations near to physiological pH in both primary and transformed keratinocytes. The inflammatory action, uterine bleeding, vascular disruption and fibrinolytic activities are enhanced with the use of copper containing IUD. ⁽¹⁴⁾ The pathway for regulation of copper in VEGF expression is similar to that utilized in hypoxia. ⁽¹⁵⁾ Thus, there are facts which suggest the role of copper in VEGF angiogenic activity. ⁽¹⁶⁾ Hence we hypothesized that elevated serum copper levels could be associated with DUB. The aim of the current study was to determine whether dysfunctional uterine bleeding was associated with elevated serum copper levels.

MATERIALS AND METHODS

This study was conducted at a rural hospital in South India.

The patient as well as the control population were drawn from the same geographical area in order to ensure that the same environmental conditions and dietary habits were prevalent for both the groups. Thirty two (n=32) patients with DUB, aged between 22-50 (mean age 36 years) years were studied, who were at different stages in the menstrual cycle based on the date of LMP: seven were in the proliferative phase and nine were in the secretory phase. For the rest of the patients, it was difficult to decide the phase, because of the prolonged bleeding of more than 20 days. The menstrual bleeding was more than seven days in duration, with increased flow and had clots present. Ultrasound studies showed absence of any other gynecological disease. The duration of excessive uterine bleeding was between 15 days-11/2 years. All patients earlier had regular (27-30 day cycles), menstruation lasting three to six days without excessive pain during menstruation. All patients had proven fertility with no previous fertility problems and they had delivered between two and five live infants with the last delivery one to three years prior to the history of DUB. They had not received any hormonal medication or used any copper containing intrauterine device for the past one year. None of these women were smokers. Women who were pregnant or who had bleeding disorders were excluded from the study. The control population consisted of age matched 30 healthy women who came for a general check up to the hospital with a history of normal menstrual cycles. There was no history of any hormonal medication or use of any copper containing intrauterine device for the past one year. None of these women were smokers or had a history of diabetes mellitus, hypertension or thyroid disorders as shown in

Table 1

Inclusion criteria

- Females of reproductive age(22-50years)
- Excessive uterine bleeding for more than 15 days
- No dysmenorrhia during menstruation
- Ultra sound scanning: No sonographic abnormalities

Exclusion criteria:

- Prolonged bleeding only in one isolated month
- Pregnant, with bleeding disorders, smokers, diabetes mellitus, hypertension or thyroid disorders

The patient's history was taken with the consent from them on a well designed proforma. The study was approved by the Institutional Review Board. The patient's consent for the study was obtained. Venous blood (5ml) was collected for estimating serum copper level and is allowed to clot for 30 min and centrifuged at 3000 revolutions per minute (rpm)

and then it is stored at -20°C Serum copper levels are estimated using a Randox kit based on 3,5 – dibromo pyridazole sulphanic acid as coloring agent(sensitivity of Randox copper levels = 1.6micromol / L or 10 microgram / dl and is linear upto 79 micromol / L or 500 microgram / dl).

RESULTS

Data was expressed as mean \pm SD. Statistical analysis was done by using unpaired t test. The odds ratio for 95% confidence limits was calculated using a 2X2 table (Simple Interactive Statistical Analysis at <http://www.quantitativeskills.com/sisa/statistics/two2hlp.htm>). Serum copper levels were significantly increased in DUB patients as compared to controls as shown in [Table 2]. Using a cut off value of serum copper level of 140 mg/dl, (mean of the control group) 75% of DUB patients and 57% of the control patients had levels greater than 140 mg/dl. Similarly, 25% of DUB patients and 43% of controls had serum copper levels less than 140 mg/dl. The odds ratio for 95% confidence limits was 2.29, thereby indicating that higher copper levels increased the risk of DUB. The kappa measurement of agreement was 0.2, indicating a fair amount of association between serum copper levels and presence of DUB.

Table: 1 Demographic detail of patients and control group

Particulars	DUB patients	Controls
Age	22-50	22-50
Duration of bleeding	15days – 11/2years	Nil
Diabetes mellitus	Nil	Nil
Hypertension	Nil	Nil
Copper Intra Uterine Device(IUD)	Nil	Nil
Ultra sound scan report	Normal	Normal
Serum copper levels	Increased	Normal
Use of copper vessels	Most of them	Two of them
Multivitamin supplement	1	nil

Table 2: Serum copper levels in women with DUB

Serum copper levels	Mean \pm SD(mg/dl)	p value
DUB patients (n=32)	235.9 \pm 149.5	<0.001
Controls (n=30)	139.7 \pm 35.6	<0.001

DISCUSSION

The source of these raised copper levels may be of dietary origin, drinking water or from use of copper cooking vessels. A specific amount of copper appears to be vital for angiogenesis to occur.^{(11),(12),(13)} Copper or copper complexes have shown to directly stimulate angiogenesis in several animal model systems while copper chelation inhibits

angiogenesis copper containing IUD increases inflammatory action and uterine bleeding and copper is found to share some of the pathways utilized by hypoxia to regulate VEGF expression^{(14),(15)}. Thus copper has a role in VEGF angiogenic activity⁽¹⁶⁾. During angiogenesis *in vivo*, before any vessel is formed the tissue to be invaded modifies its composition so as to favour growth and motility of capillary endothelium. Gullino hypothesized that copper carrying molecules might be endowed with angiogenic activity⁽¹⁷⁾. Research has shown that there is 72% increase in the copper content of malignant tumors of the ovary, uterus and cervix.⁽¹⁸⁾ Copper is also associated with the actions oestrogens. Copper accumulates in normal and neoplastic estrogen target tissues, such as uterus and mammary gland, and appears to modulate the sensitivity of these tissues to both estrogens and anti estrogens.^{(19),(20)} The ability of the metals to activate a chimeric receptor containing the hormone binding domain of Estrogen receptor suggests that their effects are mediated through the hormone binding domain.⁽²¹⁾ Increased levels of copper induce the serious toxic implications such as nausea, vomiting, hemolysis, methemoglobinemia, hepatorenal failure, chronic tubulo-interstitial nephritis, metabolic acidosis, septicemia, shock, carcinogenic effects and death in human beings⁽²²⁾. Copper has shown to influence the bioactivity or production of a number of angiogenic factors including VEGF^{(23),(24),(25)}. DUB is seen in women taking hormonal pills or reaching post menopause conditions.

From a clinical perspective, management of DUB usually involves treating the patient with hematinics to treat anaemia due to excessive bleeding, most of which contain copper helps in absorption of iron. This could lead to further elevation of copper levels which further stimulates VEGF-A leading to angiogenesis, resulting in heavier bleeding.⁽²⁶⁾ A limitation of this preliminary study was that the two groups, control and DUB, were designed based on the outcome of the disease rather than on a screening of all women exposed to dietary copper. However, a larger sample size is needed to further study the association of serum copper levels and DUB.

CONCLUSION

In conclusion, the results from this study suggest that higher levels of serum copper increases the risk of endometrial DUB. Further studies are required to understand the molecular mechanisms by which copper induced VEGF expression leads to angiogenic activity in the endometrium and raised BMI may be an additional factor which induces angiogenic activity of endometrium.

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