Original Research Article

Role of Serum copper in Dysfunctional Uterine Bleeding (DUB)

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Abstract

Introduction: DUB is one of the most frequent condition seen in gynecologic outpatient department with 10% to 15% presenting with DUB. It is characterised by anovulation and bleeding outside the regular menstrual cycle in females. Copper ions stimulate proliferation and immigration of endothelial cells in the body. **Aim of the study:** To estimate serum Copper in Dysfunctional uterine bleeding patients. **Objectives:** To estimate and compare serum Copper in endometrial thickness in biopsy and ultrasonographically proven Dysfunctional uterine bleeding patients and in controls. **Materials & Methods:** Serum Copper was estimated by using 3-5 –Di-Br-PAESA4-(3,5-Dibromo-2-pyridylazo)-N-Ethyl-N-(3-Sulphopropyl) aniline method in spectrophotometer and the levels were compared with that of controls. **Results:** In present study, serum copper levels showed high mean values in DUB cases, compared to normal controls(206.36 ± 77.08 ,151.16 ± 68.34 respectively) ($p<0.005$). **Conclusion:** Copper being associated with various angiogenic factors had higher concentration in DUB cases.

Keywords: Proliferation, Endothelial cells, HIF1α, Angiogenesis.

Introduction

Abnormal bleeding that is unrelated to pharmacological drugs, pregnancy, or identified pelvic or systemic disease is known as dysfunctional uterine bleeding (DUB). Biochemical abnormalities, increased endometrial vascular fragility, altered endometrial angiogenesis, and endothelial, epithelial inconsistencies have all been linked to DUB[1].

Endometrial repair and growth require vascularization, and the production of new vessels is dependent on interactions between hormones and growth factors. The VEGF (vascular endothelial growth factor) family and receptors have been discovered to be important regulators of angiogenesis and vascular permeability[2]. Hypoxia, growth factors, transformation, p53 mutation, oestrogen, TSH (thyroid-stimulating hormone), tumour promoters, and NO are all known to regulate VEGF gene expression (nitric oxide). HIF-1 (hypoxia-inducible factor-1) has been identified as a critical modulator of hypoxic responses. Copper has the ability to stabilise HIF-1 alpha through the suppression of prolyl hydroxylases[3]. Copper may be required for HIF-1 activation at many sites, including HIF-1alpha production, stabilisation, transport from the cytoplasm to the nucleus, binding to the HRE sequence of target genes (VEGF), and assembly of the HIF-1 transcriptional complex[4].

Increased buildup of HIF 1 alpha in the presence of hypoxia is thought to lead to angiogenesis in Dysfunctional uterine haemorrhage patients[5].

Aim of the study

To estimate serum Copper in Dysfunctional uterine bleeding patients

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Objectives

To estimate and compare serum Copper in endometrial thickness in biopsy and ultrasonographically proven Dysfunctional uterine bleeding patients and in controls.

Materials & Methods

It was case-control study conducted from July 2011 – December 2013 at Department of Biochemistry, Kasturba Medical College & Hospital (KMC), Manipal. The study was carried out after obtaining approval from the institutional ethics committee of KMC Manipal. The study was carried out after obtaining informed written consent from DUB cases and controls.

Sample size was calculated by using this formula

Sample size calculation:

$n = 2[(Z_\alpha + Z_\beta) S]^2/2 [d]^2 = 45$

$Z\alpha$ = value at specified confidence level

Z_β = value at specified power

S = pooled standard deviation of observations of 2 samples

d = clinically significant difference

In the present study, 40 DUB cases group and 40 subjects as normal control group was taken. (Total 80 subjects)

Nonpregnant women between 18-45 years of age with irregular, excessive uterine bleeding continuously for more than 3 months, with previous regular (27-30 days) menstrual cycles lasting for 3-6 days, patients who have given live births with the last delivery being 1-3 years prior to history of DUB, patients who have not received any hormonal medication or used copper IUCD for past 1 year were included as DUB cases. Healthy, Age matched, Non pregnant women, with the history of normal menstrual cycle, not on hormonal medication or used copper IUCD for past 1 year were included as controls. Patients with other gynaecological diseases like Fibroid, Polyp, tumors, smokers, having thyroid disorders, bleeding disorders, having diabetes, hypertension, High BMI, Tuberculosis, on aspirin therapy, patients who received any hormonal medication or used copper IUCD for past one year were excluded from the study.

A random venous blood sample (4 mL) was drawn each from cases and controls into a sterile red topped vacutainer & was allowed to clot for 30 minutes. The sample was centrifuged at 1900 rotations per minute for 20 minutes and the serum was separated and collected in 3 different aliquots. It was stored at -20°C until analysed.

Serum Copper was estimated by using 3-5 –Di-Br-PAESA4-(3,5-Dibromo-2-pyridylazo)-N-Ethyl-N-(3-Sulphopropyl) aniline method in spectrophotometer. Principle of the test is, at pH 4.7, Copper which is bound to ceruloplasmin, is released by a reducing agent. It then reacts with a specific colour reagent, 3-5 –Di-Br-PAESA4-(3,5-Dibromo-2-pyridylazo)-N-Ethyl-N-(3-Sulphopropyl) aniline, to form a stable coloured chelate. The intensity of the colour is directly proportional to the amount of copper in the sample[6].

Results

In the present study, angiogenic parameters copper were assessed in serum so as to examine angiogenesis as a presumed cause of DUB and to prove these angiogenic parameters as early noninvasive biomarkers of DUB. The data was statistically analyzed using SPSS software version 14. Significance of difference between DUB cases and normal controls observed was assessed by using the unpaired student 't'test. ANOVA test was applied to compare parameter between more than two groups. Pearson's correlation was used to correlate the various parameters.

Table 1: Demographic pattern of normal controls and DUB cases

Groups	Age in years (Mean ± SD)	Age in years (Range)
Controls (n=40)	33.9 ± 7.10	22-45
Cases (n=40)	38.8 ± 5.32	28-45

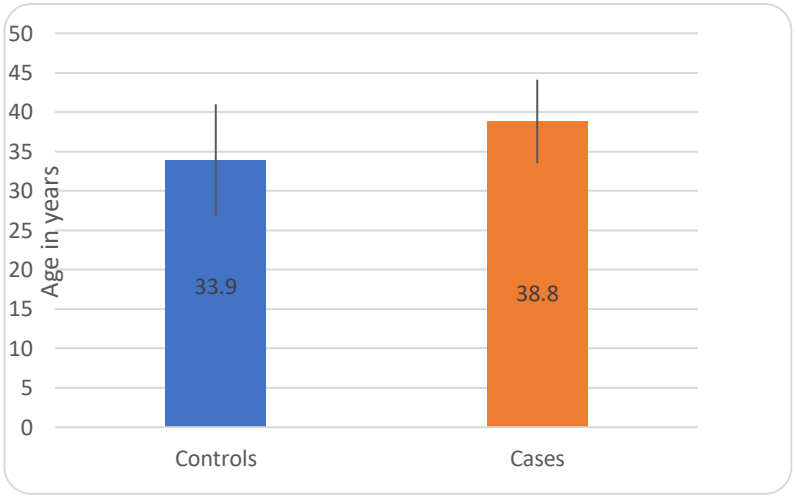

Fig. 1: Bar chart showing mean age of controls and DUB cases

Serum copper levels were significantly elevated in DUB cases compared to controls (p<0.005) as shown in table 2.

Table 2: Levels of serum copper in controls and DUB cases

Groups	Serum copper (µg/dL) (Mean ± SD)	p value	Serum copper (µg/dL) Range
Controls(n=40)	151.16±68.34	<0.005	46-324
Cases(n=40)	206.36±77.08		95.13 - 362

p< 0.005 statistically significant

Discussion

The goal of this research was to learn more about the role of serum copper in endometrial angiogenesis. Although copper's effect in different malignancies has been extensively established, its impact in DUB has not been investigated. The investigation was necessary since DUB is a prevalent gynaecological disease, and the genesis of this disorder was unknown.

In this investigation, serum copper levels in DUB patients were higher than normal controls (206.36 77.08, 151.16 68.34, respectively) (p0.005). This conclusion was in line with that of Rafi A et al[7], who found that DUB patients had higher serum copper levels than normal controls (155.94 0.92, 104.08 11.43, respectively) (p0.0001). In the treatment of DUB, haematinics are frequently prescribed to address anaemia caused by profuse bleeding. Copper is present in the majority of haematinics, as copper aids in iron absorption. Excess copper, on the other hand, may cause further elevations in serum copper levels, as well as severe effects on the endometrium in DUB patients.

Investigators Dabek JT et al[8],Chan A et al[9], Poo JL et al[10], Vaidya SM et al[11] found substantial increases in mean total serum copper concentrations in all patient groups, including gynaecological cancer, breast cancer, lung cancer, and gastro-intestinal cancer (p 0.05).According to the recognised standard range of serum copper levels (80-155g/dL), they were increased in 64.4 percent of DUB patients and 35.6 percent of the normal control group in the current investigation. Elevated copper levels in the normal control group could be attributable to metal exposure mostly via dietary sources such as food and water, air, and dining behaviours in copper-containing vessels. Copper deficiency is frequently linked to cancer and may be a risk factor for estrogen-dependent malignancies.

Copper boosted factors involved in artery development and maturation, such as vascular endothelial growth factor (VEGF), which was primarily responsible for its angiogenesis effect, according to Xie H et al[12].The activation of hypoxia-inducible factor-1 (HIF-1), a key transcription factor that controls VEGF expression, required copper. HIF-1 activated multiple important genes involved in cellular metabolism and function, and it was only one of the genes regulated by it. It was comprehensible that copper stimulation of vascularization

was superior than the effect of VEGF alone since copper was required for HIF-1 activation. Harris ED [(14)], in a study done by Leone N et al.[13] found that copper increased angiogenesis via the VEGF signalling pathway[14].

The current study found that serum copper is important in the mechanisms of dysfunctional uterine haemorrhage, which could lead to new diagnostic and treatment possibilities.

Conclusion

In comparison to normal controls, DUB cases had higher serum copper levels (p0.05). According to the established reference range (80-155g/dL), increased serum copper levels were found in 64 percent of DUB patients and 35.6 percent of normal controls. It was established that copper was linked to angiogenic factors such as HIF1; however, because normal controls had lower levels of angiogenic factors, increased copper did not stimulate angiogenesis, and normal controls did not have excessive bleeding.

References

1. Zhang X , Qi C , Lin J . Enhanced expressions of matrix metalloproteinase (MMP)-2 and -9 and vascular endothelial growth factors (VEGF) and increased microvascular density in the endometrial hyperplasia of women with anovulatory dysfunctional uterine bleeding. Fertil Steril 2010; 93(7):2362-7.
2. Erdem O , Erdem M , Erdem A , Memis L , et al. Expression of vascular endothelial growth factor and assessment of microvascular density with CD3 and endoglin in proliferative endometrium, endometrial hyperplasia, and endometrial carcinoma. Int J Gynecol Cancer 2007; 17(6):1327-32.
3. Smith SK. Regulation of angiogenesis in the endometrium. Trends Endocrinol Metab 2001; 12 (4):147-51.
4. Takahashi H, Shibuya M. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. Clin Sci (Lond) 2005; 109(3):227-41.
5. Laughner E, Taghavi P, Chiles K, Mahon PC, et al. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001; 21 (12):3995-4004.
6. Fayez JA. Dysfunctional uterine bleeding. Am Fam Physician1982; 25(3):109-15.
7. Rafi A, Ramkrishna D, Sabitha K, Mohanty S, et al. Serum copper and vascular endothelial growth factor (VEGF) in dysfunctional uterine bleeding. American Journal of Biochemistry and Molecular Biology 2011; (3):284-90.
8. Dabek JT, Hyvönen-Dabek M, Härkönen M, Adlercreutz H. Evidence for increased non-ceruloplasmin copper in early-stage human breast cancer serum. Nutr Cancer 1992; 17(2):195-201.
9. Chan A, Wong F, Arumanayagam M Serum ultrafiltrable copper, total copper and caeruloplasmin concentrations in gynaecological carcinomas. Ann Clin Biochem 1993; 30:545-9.
10. Poo JL, Romero RR, Robles JA, Montemayor AC, et al. Diagnostic value of the copper/zinc ratio in digestive cancer: a case control study. Arch Med Res 1997; 28(2):259-63.
11. Vaidya SM, Kamalakar PL. Copper and ceruloplasmin levels in serum of women with breast cancer. Indian J Med Sci 1998; 52(5):184-7.
12. Xie H, Kang YJ. Role of copper in angiogenesis and its medicinal implications. Curr Med Chem 2009; 16(10):1304-14.
13. Leone N, Courbon D, Ducimetiere P, Zureik M. Zinc, copper, and magnesium and risks forall-cause, cancer, and cardiovascular mortality. Epidemiology 2006; 17(3):308-14.
14. Harris ED. A requirement for copper in angiogenesis. Nutr Rev 2004; 62(2): 60-4.

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