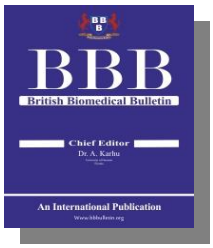
Journal Home Page www.bbbulletin.org

BRITISH BIOMEDICAL BULLETIN



Original

Investigation of Serum Copper and Iron Deficiency Anaemia In Patients with Abnormal Uterine Bleeding

Yousef Rezaei Chianeh*[1], Pooja R Shenoy[1], Azadeh Bagheri[1],Ullas Kamath[2], Lavanya Rai[3], Pratap Kumar[3] and Pragna Rao[1]

[1]Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal - 576104, Karnataka, India
[2]Melaka Manipal Medical College, Manipal University, Manipal - 576104, Karnataka, India
[3]Department of Obstetrics & Gynecology, Kasturba Medical College, Manipal University, Manipal - 576104, Karnataka, India

ARTICLE INFO

Received 12 Aug. 2015
Received in revised form 30 Aug. 2015
Accepted 10 Sep. 2015

Keywords:
Menorrhagia,
Abnormal Uterine Bleeding (AUB),
Copper,
Haemoglobin (Hb).

Corresponding author: Department of Biochemistry,
Kasturba Medical College, Manipal University, Manipal, Karnataka, India

E-mail address:
y.rezaei1980@gmail.com

ABSTRACT

Aim: To investigate the association and pathophysiology of copper in anaemic patients suffering from abnormal uterine bleeding.
Materials and Methods: The present case control study was undertaken in the Departments of Biochemistry and Obstetrics & Gynaecology, Kasturba Medical College, Manipal University, Manipal, India. In this study 120 patients were included, aged between 18-52 years with a history of excessive menstrual bleeding for more than 3 months.
Sample size: This study was done in 120 female with a history of abnormal uterine bleeding and 120 controls (women with regular menstrual cycle). Abnormal uterine bleeding was diagnosed based on history of bleeding and haemoglobin concentration. Copper was estimated using 3, 5-dibromo-2-pyridylazo-N-ethyl-N-3 sulphopropyl aniline. Endometrial thickness was obtained from USG. Hemoglobin was estimated using Drabkin's method.
Main outcome measures: The level of serum copper measured and the correlation coefficient is applied with haemoglobin to investigate a probable association of copper and haemoglobin in patients with abnormal uterine bleeding.
Results: The serum copper concentration in patients was 239.55(189.236, 298.497) µg/dl (median IQR) and in control was found to decrease 109.39(91.262, 131.4) µg/dl (median IQR) which is statistically significant $p<0.001$. Haemoglobin concentration was decreased in abnormal uterine bleeding patients 9.29±1.65 g/dl when compared with control 12.2 ± 0.87 g/dl which is statistically significant $p<0.001$. Endometrial thickness in patients was 11.43±4.05 mm and in controls was found to decrease 7.7±2.33 mm. The correlation coefficient was plotted between copper and haemoglobin in both cases and controls and weak negative correlation found between these two parameters.
Conclusion: Increase serum copper might be seen as predictors of abnormal uterine bleeding and investigation of serum copper could increase efficacy of treatment.

Introduction

Gynaecological health is an important component of any woman's health status. Gynaecological disorders can have a substantial impact on many aspects of quality of life, including reproductive ability, sexual functioning, mental health, and the ability to work and to perform routine physical activities[1,2].

About 30% of women seek medical assistance for AUB during their reproductive age group and about one third of hysterectomies are carried out for AUB alone. AUB ranks next to the abnormal vaginal discharge among the total gynaecology OPD attendance.

The menorrhagia, which is excessively heavy menstrual bleeding in the absence of a well defined pelvic pathology, is termed as Abnormal Uterine Bleeding (AUB). The probable cause of AUB is due to tumors of the ovary, uterus, and cervix, introduction of copper containing Intra uterine Device (IUD) or ovulatory dysfunction due to failure of normal progression in cyclic hormonal stimulation of endometrium[3].

Understanding of the cellular mechanism that lead to AUB 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 AUB. Thus, the link between hormones and AUB is not direct[4,5].

Endometrial angiogenesis that controlled by angiogenic factors are involved in pathogenesis of AUB if it is uncontrolled. The principle angiogenic factors that involved in endometrial blood vessel formation are VEGF. The mechanism by which VEGF regulates the endometrial blood vessel formation in every month after shedding of the endometrium is not completely explained[6,7].

A certain amount of copper appears to be vital for angiogenesis to occur[8-10]. Copper sulfate induces VEGF expression at 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[11]. The pathway for regulation of copper in VEGF expression is similar to that utilized in hypoxia[12]. Thus, there are facts which suggest the role of copper in VEGF involvement in angiogenesis[13]. Hence we hypothesized that elevated serum copper levels could aggravate AUB condition. The aim of the current study was to determine whether abnormal uterine bleeding was associated with elevated serum copper levels and to correlate a serum copper concentration with haemoglobin and to find out a possible effect of copper toxicity on pathology of AUB.

Materials and methods

A case control study was conducted in the Department of Biochemistry and Obstetrics & Gynecology at Kasturba Medical College, Manipal University, Manipal, from May 2013 to January 2015. The study protocol received approval by Institutional Ethic committee. All women with abnormal uterine bleeding attending outpatient department (OPD) of Obstetrics and Gynecology were offered voluntary participation in the study after written informed consent.

This study was done in 120 female with a history of abnormal uterine bleeding and 120 controls (women with regular menstrual cycle). The patients with AUB

were recruited and subjected to detailed questionnaire about patterns of abnormal bleeding, significant medical and surgical history in the past and intake of any drugs or hormonal preparation including details of prior treatment if administered. The control group selected among women with regular menstrual cycle. Patients with AUB were examined and graded according to FIGO classification system (PALM-COIN). Only patients who belonged to the endometrial (AUB-E) category were selected for the study[14].

Copper was estimated using 3, 5-dibromo-2-pyridylazo-N-ethyl-N-3 sulphopropyl aniline. Hemoglobin was estimated using Drabkin's method.

Results

To see the correlation between copper and haemoglobin and to investigate the effect of copper toxicity on severity of AUB, we have studied 120 AUB patients aged between 18-52 (Group I) against 120 age matched non-pregnant healthy women with a regular menstrual cycle aged between 19-45 (Group II). Demographic representation of patient's data illustrated in table 1 and 2.

We observed a higher serum copper concentration in AUB patients 239.55(189.236, 298.497) µg/dl median IQR ($P<0.001$) when it is compared to control group 109.39(91.262,131.4) µg/dl median IQR ($P<0.001$). Haemoglobin is decreased significantly in patients, 9.29±1.65 g/dl mean±std ($p<0.001$). The correlation coefficient between copper and haemoglobin in patients and control group are illustrated in figure 1, 2 and table 3. The correlation coefficient between serum copper and haemoglobin in both cases and controls shows a weak negative correlation that shows decrease haemoglobin concentration is mainly due to heavy menstrual bleeding rather than copper toxicity effect but oxidative stress inducing activity of higher serum copper concentration should not be neglected.

The uncontrolled endometrial angiogenesis in AUB patients could be due to increase copper concentration and may result in expression of angiogenic factors, hence enhances endometrial blood vessel formation and endometrial growth that could manifest in increase endometrial thickness (ET).

Discussion

In this study, we found a weak negative correlation between copper and haemoglobin but we believe that copper plays an important role in regulating endometrial angiogenesis, the lower haemoglobin concentration is due to heavy menstrual blood loss but anaemic condition may be aggravated in copper toxicity through dysregulation of endometrial blood vessels formation and increase fragility of vessels.

The source of increase in serum copper concentration might be of dietary sources, drinking water or from the use of copper cooking vessels[15].

A specific amount of copper appears to be important for angiogenesis to occur. 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[16]. Thus copper has a role in VEGF angiogenic activity. During angiogenesis in vivo, before any blood vessel formation the tissue to be invaded modifies its composition so as to favour growth and motility of capillary endothelium. It has been hypothesized that copper carrying molecules might be endowed with angiogenic activity[17].

Research has shown that there is a 72 % increase in the copper content of malignant tumors of the ovary, uterus and cervix[17]. Copper is also associated with the oestrogens actions. 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[23-25]. AUB is seen in women taking hormonal pills or reaching post menopause conditions. Management of AUB usually involves treating the patient with hematinics to treat anaemia due to excessive blood loss, 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]. The limitation of this study was that the two groups, control and AUB, 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 AUB.

Conclusion

The results from this study suggest that higher levels of serum copper may even deteriorate the risk of endometrial AUB. Further studies are required to understand the molecular mechanisms by which copper involvement in VEGF expression leads to angiogenic activity in the endometrium and may be an additional factor which induces angiogenic activity of endometrium.

Acknowledgement

We thank all women who participated in this study and the staffs and nurses of Department of Obstetrics and Gynaecology of Kasturba Medical College, Manipal University for their help with recruitment.

Conflict of interest

The authors have no conflicts of interest to declare.

References

1. Carlson KJ, Miller BA, Fowler FJ Jr. The Maine women's health study: I. outcomes of hysterectomy. Obstet Gynecol. 1994;83:556-565.
2. Carlson KJ, Miller BA, Fowler FJ Jr. The Maine women's health study: II. Outcomes of nonsurgical management of leiomyomas, abnormal bleeding,and chronic pelvic pain. Obstet Gynecol. 1994;83:566-572.
3. Khandhadiya P K,Yousef Rezaei Chianeh, R.Pragna. "Role of serum copper and ceruloplasmin level in patients with dysfunctional uterine bleeding." *Int J Reprod Contracept Obstet Gynecol*.2014;3(2):333-4.
4. P.A.W.Rogers, F.Martinez, J.E.Girling, *et al*, Influence of different hormonal regimes on endometrial microvascular density and VEGF expression in women suffering from breakthrough bleeding; Human Reproduction. 2005; 20: 3341-47.
5. Chianeh YR, Rao P. Molecular and hormonal regulation of angiogenesis in proliferative endometrium. *Int J Res Med Sci*. 2014;2:1-9.
6. Gargett CE, Lederman F, Heryanto B, Gambino LS and Rogers PAW. Lack of correlation between VEGF production and

angiogenesis in the human endometrium; Human Reproduction.1999; 14: 2080-88.
7. Gargett CE, Lederman F, Heryanto B, Gambino LS and Rogers PAW. Focal vascular endothelial growth factor correlates with angiogenesis in human endometrium; Human Reproduction.2001; 16: 1065-75.
8. Pan Q, Kleer CG, Van Golen KL, *et al*., Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis; Cancer Research.2002; 62:4854-9.
9. Yoshii J, Yoshiji H, Kuriyama S, *et al*., The copper chelating agent, trientine, suppresses tumor development and angiogenesis in the murine hepatocellular carcinoma cells; *International Journal of Cancer*.2001; 94: 768-73.
10. Brem SS, Zagzag D, Tsanaclis AM . Inhibition of angiogenesis and tumor growth in the brain. Suppression of endothelial cell turnover by penicillamine and the deletion of copper, an angiogenic cofactor; *American Journal of Pathology*.1990; 137:1121-42.
11. Kulier R, F.M. Helmerhorst, P. O'Brien, M. Usher- Patel and C.D'Arcangues. Copper containing framed intra-uterine devices for contraception; Cochrane database of systematic reviews, 2007, Issue 4.CD005347 – CD005347.
12. Bagheri A, Chianeh YR, Rao P. Role of angiogenic factors in recurrent pregnancy loss. *Int J Reprod Contracept Obstet Gynecol*. 2013;2:497-502.
13. Bagheri A, Chianeh YR, Kumar P, Rao P. Angiogenic factors in relation to embryo implantation. *Int J Reprod Contracept Obstet Gynecol*. 2014;3:872-9.
14. Munro, M. G., Critchley, H. O., Broder, M. S., Fraser, I. S., & FIGO Working Group on Menstrual Disorders. "FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age." *International Journal of Gynecology & Obstetrics*. 2011;113.1: 3-13.
15. Chianeh RY, Rao P. Role of copper and vascular endothelial growth factor (VEGF) on endometrial angiogenesis. *J Krishna Institute of Medical Sciences University*. 2013;2(2):6–17.
16. Sen CK, Khanna S, Venojarvi M, *et al*., Copper induced vascular endothelial growth factor expression and wound healing; *American Journal of Physiology Hear Circulation Physiology*. 2002; 282:H1821-27.
17. Cantarella, Giuseppina, Laurence Lempereur, Marco Presta, Domenico Ribatti, Gabriella Lombardo, Philip Lazarovici, *et al*. "Nerve growth factor–endothelial cell interaction leads to angiogenesis in vitro and in vivo." *The FASEB Journal*. 2002; 16.10: 1307-1309.
18. Margalioth, E.J.,J.G.Schenker and M. Chevion. Copper and zinc levels in normal and malignant tissues; Cancer. 1983; 52: 868 – 872.
19. Fuchs,A.G., R.Mariotto, E.S.de Lustig. Serum and tissue copper content in two mammary adenocarcinomas with different biological behaviour; *European Journal of Cancer Clinical Oncology*.1986; 22: 1347 - 1352.
20. Schwartz, A.E.,G.W.Leddicotte, R.W. Fink and E.W. Friedman. Trace elements in normal and malignant human breast tissue; Surgery.1974; 76: 325 – 329.
21. Martin, M.b., R. Reiter, T.pham, *et al*. Estrogen like activity of metals in MCF-7 breast cancer cells; Endocrinology.2003; 144: 2425 – 2436.
22. Almansour, M.I. Biochemical effects of copper sulfate, after chronic treatment in quail; *Journal of Biolological science*.2006; 6(6):1077 – 1082.
23. Chandana K. Sen, Savita Khanna, et.,al., Copper induced vascular endothelial growth factor expression and wound healing ; *American Journal of Physiology Heart Circulation Physiology*. 2002;282: H1821-27.
24. Harris Edward D. A requirement for copper in angiogenesis; Nutrition Reviews. 2004;62:60-4.
25. Lydia Finney, Suneeta Mandava, Lyann Ursos, et.,al. X-ray fluorescence microscopy reveals large-scale relocalization and extracellular translocation of cellular copper during angiogenesis.; PNAS. 2007; 104:2247-52.

26. Usama M Fouda, Dalia Yossef, Hassan M Gaafar. Uterine artery blood flow in patients with copper intrauterine device-induced abnormal uterine bleeding; *Middle East Fertility Society Journal*.2010; 15: 168-173.

Table 1: Demographic details of haemoglobin and age of the AUB patients and controls

	Patients		Controls		P value
Parameters	Range	Mean ± std	Range	Mean ± std	
Age (years)	18-52	39.25±7.77	19-45	34.14±7	<0.001
Haemoglobin (g/dl)	5.20-12.00	9.29±1.65	9.50-14.20	12.2±0.87	<0.001
Endometrial thickness (ET) mm	4.3-30	11.43±4.05	3.5-12	7.7±2.33	<0.001

When the result compared between AUB patients and controls, Haemoglobin concentration was significantly decreased and statistically were significant (P<0.001). Endometrial thickness was found to increase in patients and it was statistical significance when compared between patients and controls p<0.001.

Table 2: Demographic details of serum copper of the AUB patients and controls

	Patients		Controls		P value
Parameters	Range	Median IQR	Range	Median IQR	
Serum Copper (µg/dl)	58.8-504.52	239.55(189.236,298.497)	34.75-352.8	109.39(91.262,131.4)	<0.001

Serum copper is expressed in median with IQ range since there were a large variation between copper value in individual patients and serum copper concentration is found to increase in patients when it is compared with healthy control group (women with regular menstrual cycle). The variation in copper concentration between cases and controls were statistically significant (p<0.001).

Table 3: The correlation coefficient between copper and Haemoglobin in AUB patients and controls

Group	Parameter		Haemoglobin
Cases	Copper	R	-0.118
		P	0.201
		N	120
Controls	Copper	r	-0.055
		P	0.551
		N	120

r=correlation coefficient P=significance N=number of participant

The correlation coefficient is applied between copper and haemoglobin in both patients and controls. A weak negative correlation was observed in both cases (r= -0.118) and control group (r= -0.055).

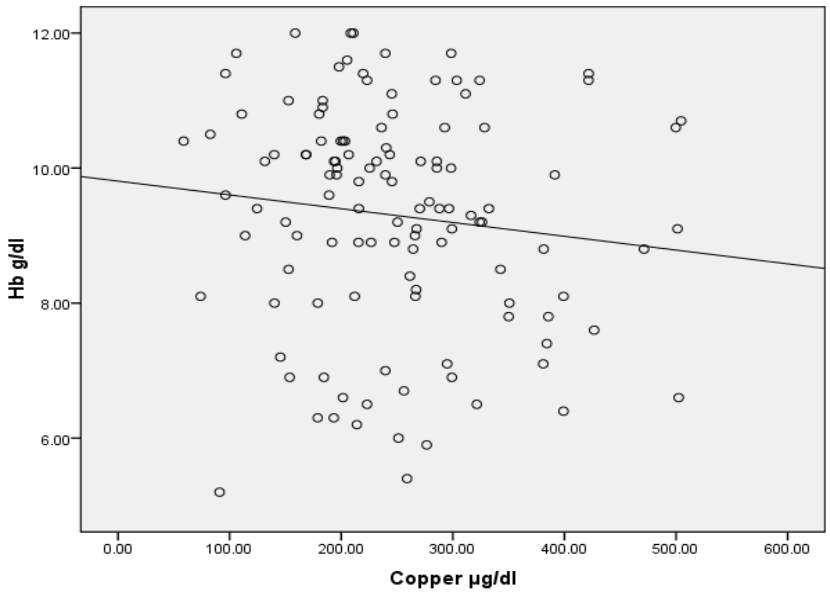


Figure 1: Correlation coefficient between copper and Haemoglobin in patients with abnormal uterine bleeding.

This figure illustrates a correlation between copper and haemoglobin among cases (AUB). Weak negative correlation is observed between these parameters.

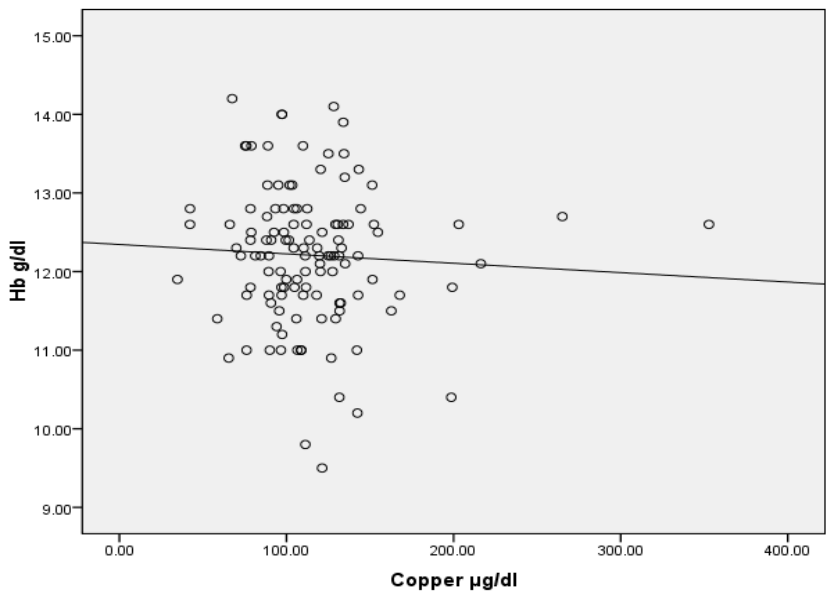


Figure 2: Correlation coefficient between the copper and haemoglobin in control group.

This figure illustrates a correlation between copper and haemoglobin among controls. Weak negative correlation is observed between these parameters.