Biomedical Research 2022; 33 (4): 113-118

ISSN 0970-938X
www.biomedres.info

Impact on certain essential trace elements in polycystic ovarian syndrome patients in north-west Indians.

Kuldip Singh*

Department of Biochemistry, Government Medical College-Patiala, Punjab, India

Abstract

Background: Polycystic Ovary Syndrome (PCOS) is an endocrine disorder that affects 6%-7% of premenopausal women. Ferritin is a ubiquitous intracellular protein that is essential for the regulation of iron homeostasis. Iron is a strong pro-oxidant and high body iron levels are associated with an increased level of oxidative stress, pathogenesis of various disease like Diabetes, Cardiovascular diseases, PCOS etc.

Aim: The present study was designed to evaluate certain essential trace elements like Ferritin, Copper, Magnesium, Zinc, Selenium levels in young PCOS women's from tertiary care hospitals of Punjab state of India.

Research Design and Methods: 35 PCOS patients were recruited from the urban and rural area of the Punjab State in the age range of 20 to 40 years.

Results: Serum ferritin ($P \leq 0.001$) and copper ($P \leq 0.05$) concentrations were significantly increased while a significant fall in zinc ($P \leq 0.05$), magnesium ($P \leq 0.05$) and selenium ($P \leq 0.01$) was observed in women presenting with PCOS with respect to healthy control subjects.

Conclusion: A significant increase in ferritin and Cu while a significant fall in Se, Zn and Mg levels observed in the present study suggesting that these essential trance elements are an important etiological role in the pathogenicity and increased complication of PCOS.

Keywords: Polycystic Ovary Syndrome, Ferritin (Fe), Copper (Cu), Magnesium (Mg), Zinc (Zn), Selenium (Se), Oxidative Stress.

Accepted on April 24, 2022

Introduction

Polycystic ovary syndrome is a complex multifactorial endocrinopathy affecting a substantial population of reproductive aged women and is the most common cause of infertility. Genetics and lifestyle may develop the main features of PCOS. Women diagnosed with PCOS are usually presented with hyper androgenism, ovulatory dysfunction, and polycystic ovaries [1]. The main features of PCOS include androgenic features, menstrual dysfunction, and polycystic ovaries. PCOS diagnosis according to the National Institute of Health (NIH) consensus necessitates the existence of oligo- or amenorrhea and hyperandrogenaemia without a known disorder that explain the cause of hyperandrogenaemia. The Rotterdam criteria define PCOS by the presence of two of the three following features: hyperandrogenaemia, oligo-or amenorrhea and polycystic ovaries by ultrasound imaging [2,3].

Trace Elements have important role in PCOS. Many trace elements are important for optimum human metabolic function. These micronutrients serve a variety of functions including catalytic, structural and regulatory activities in which, they interact with macromolecules such as enzymes, prohormones, and biological membrane receptors [4]. Others play a crucial role in the immune system Trace elements are uniquely required for maintenance of life and health. Lack or inadequate supply of such nutrients produces a functional impairment or can result in disease [4]. Numerous studies had revealed the role of increment of oxidative stress which could result from excessive production of reactive oxygen species in pathogenesis of polycystic ovary [4,5]. Over production of Responsive Oxygen Species (ROS) is a typical component in women with polycystic ovary. Selenium is involved in many biological functions, such as, protection against oxidative stress, immune function and thyroid function [6]. Selenium

is most widely recognized as a substance that speeds up the metabolism of fatty acids and works together with vitamin E (Tocopherol) as antioxidant. Selenium also appears to work as an anti-inflammatory agent in certain disorders [7,8].

Copper, zinc and manganese are fundamental micronutrients which joined into numerous proteins and metalloenzymes and they are dependable in cell metabolic system and oxidative stress pathways which may contribute to oxidative stress [9,10]. Cu catalyzes the synthesis of highly Reactive Oxygen Species (ROS) that causes oxidative damage to proteins, lipids, DNA and other molecules, Copper has an antioxidant action that protect cells from damage and is also a component of many enzymes that is responsible for the release of energy from carbohydrates, fat and protein. Copper is also important for formation of red blood cells, bone and connective tissues. PCOS may result in dysregulation of systemic copper homeostasis. Another element of biological effect is magnesium and is essential to good health. As the second most abundant intracellular cation in the human body, magnesium is involved in more than 300 ATP and kinase dependent enzymatic reactions. Magnesium plays an essential physiological role in many functions of the body. It may play a role in glucose homeostasis, insulin action in peripheral tissues, and pancreatic insulin secretion, magnesium functions as a cofactor for several enzymes critical for glucose metabolism utilizing high energy phosphate bonds [8-10]. Zinc is directly involved in the synthesis, storage and secretion of insulin, as well as conformational integrity of insulintaste acuity and enhances the *in vitro* effectiveness of insulin. The function of zinc in the body metabolism is based on its enzymatic affinity and way of a zinc-enzyme complex or metalloenzymes. Zinc is required for insulin synthesis and storage and insulin is secreted as zinc crystals. It maintains the structural integrity of insulin. Zinc deficiency may play a role in the pathogenesis of polycystic ovarian syndrome and may be related with its long-term metabolic complications [11].

Zinc is an essential trace element required as a structural, catalytic, and regulatory ion for many enzymes, proteins and transcriptional factors activities. As a result, zinc plays an important role in many homeostatic response of the body (e.g. oxidative stress) and in many biological functions (e.g. immune deficiency). Zinc also demonstrated multiple roles as a modulator of inflammation in cell cultures and animal model. Zinc supplementation improves inflammatory reaction in PCOS patients [12]. Copper is an essential trace element that is important to the proper functioning of organs and metabolic processes. Copper has an antioxidant action that protects cells from damage, and is also a component of many enzymes that is responsible for the release of energy from carbohydrates, fat and protein. Copper is also important for formation of red blood cells, bone and connective tissues. PCOS may result in dysregulation of systemic copper homeostasis. Mg has been identified as a necessary nutrient for energy production and synthesis of nucleic acids. Selenium plays a significant vital role in the undisturbed functioning of the reproductive system which proves to the correlations between the Se intake and the fertility as well as disorders of procreation processes [12,13]. So, the present study was designed to evaluate the impact on certain trace elements such as ferritin, zinc, copper, selenium, magnesium levels.

Materials and Methods

The present study was carried out on 35 of PCOS patients. These PCOS women patients were recruited from both rural and urban areas of Punjab State. PCOS women were recruited based on the diagnostic criteria as per 2003 Rotterdam Revised Consensus Meeting i.e. Oligomenorrhea, Clinical or Biochemical Hyperandrogenemia and the presence of polycystic ovaries [3]. Each recruited participant had at least two symptoms of the disease were considered as PCOS patient. The subjects with conditions like prolactinoma, congenital adrenal hyperplasia, Cushing's syndrome and virilizing ovarian or adrenal tumors were excluded from the study and 35 healthy subjects in the control group were selected from a population with the same socioeconomic condition who referred to the clinic for check-up and had normal ovulating cycles and no signs of hyperandrogenism. Ultrasonography confirmed that these women did not have clinical features of hyperandrogenism or PCOS. They were matched for age and Body Mass Index (BMI) to the affected cases. The subjects in the control group didn't have any systemic diseases, and they didn't use any medications that might affect their reproductive physiology or iron status. All people with anemia (Hemoglobin concentration of less than 12 g/dl) were excluded from the study.

Inclusion criteria

Inclusion criteria were all the patients attend PCOS clinic of infertility OPD with following criteria age 20-40 years and diagnosed as PCOS according to the 2003 Rotterdam revised consensus meeting.

Exclusion criteria

Exclusion criteria were recent H/O blood transfusion or intake of iron, history of heavy menstrual bleeding, diabetes, known chronic inflammatory conditions e.g.; chronic cough, asthma, tuberculosis and anemia: hemoglobin <11 g/dl was excluded, which is frequently associated with iron deficiency.

All subjects recruited for the study were vegetarian, non-smokers and non-alcoholic, with no positive family history of non-communicable chronic diseases like diabetes, Cardiovascular Diseases (CVD). Data was obtained from subjects using an interviewer-based questionnaire where the detailed information on their lifestyle, medical history, diet etc. and after obtaining the written consent these were considered for the study. Blood Pressure was measured 3

times every 2 minutes after an initial 10-minute rest period to the nearest of 2 mm Hg in the sitting position, using a mercurial sphygmomanometer and appropriately sized cuffs.

Study design

This study was a case control cross-sectional prospective study. This study protocol was approved by the Institution Ethical Committee. A detailed history, physical, and systemic examination, including measurement of height, weight, heart rate, blood pressure in the sitting position, Body Mass Index (BMI), fasting glucose along with HbA1C and certain trace elements such as Ferritin, Zinc, Copper, Magnesium was carried out in every subject who entered the study as per a pre-designed performa for assessing the signs of diabetes, heart failure and also the presence of any exclusion criteria.

Data collection

Measurements of anthropometric parameters: Height (m) and Weight (kg) were measured while subjects were wearing light clothing without shoes. Blood Pressure (BP) was measured using an automatic manometer with an appropriate cuff size on the right arm after a resting period of 5 min.

Specimen collection, specimen (serum/plasma) preparation and storage: A volume of 5 ml of peripheral venous blood was collected by vein puncture using a dry, disposable syringe between 8 and 9 AM after an overnight fast from both groups (Control and PCOS subjects) in potassium oxalate and sodium fluoride vial (2 ml) and plain (3 ml) vial. The blood of potassium oxalate and sodium fluoride vial was immediately centrifuged at 4000 rpm for 10 minutes for the preparation of plasma and the blood collected in plain vial was kept at 37ºC for half an hour after that the blood samples were centrifuged at 4000 rpm for 15 minutes for the separation of serum. The plasma and serum was used for various biochemical assays as under:

Biochemical assays

Estimation of plasma glucose levels: Fasting plasma glucose levels were estimated spectrophotometrically using an enzymatic test kit based on GOD-POD method supplied by Transasia Biomedical Private Limited, Mumbai (India).

Estimation of glycosylated Hemoglobin (HbA1C): HbA1C was analyzed by using kit supplied by Transasia Bio-Medicals Ltd. Solan (HP) in Technical collaboration with ERBA agnostic Mannheim, Germany based onion-exchange resin method in which a hernolyzed preparation of the whole blood is mixed continuously for 5 ruin with a weak binding cation exchanges resin. During this time, non-HbAlc binds to the resin. After the mixing period a filter is used to separate the supernatant containing the glycohemoglobin from the resin. The glycohemoglobin percent is determined by measuring the absorption at 415 nm of the glycohemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percentage glycohemoglobin.

Estimation of trace elements: The trace elements such as Ferritin, Zinc, Copper, Magnesium and selenium in serum was estimated spectrophotometrically using a standardized test kit supplied by Transasia Biomedical Private Limited, Mumbai (India) by colorimetric method in ERBA-XL-360 fully autoanalyzer.

Statistical analysis

The data was expressed as Mean ± SD and analyzed with the SPSS 16.0.7 statistical software package. Differences between the obese and control subjects were evaluated using the Student's independent samples "t" test. Differences were considered statistically significant at $P<0.05$.

Results

Anthropometric parameters: The Anthropometric measurements of both type-2 diabetic patients and normal healthy control subjects are summarized in the Table 1. The body weight, height, BP systolic and BP-diastolic was 83.48 ± 5.11 kg, 172.02 ± 5.21 cm, 120.27 ± 3.19 mmHg and 98.99 ± 3.78 mmHg respectively in PCOS patients w. r. t. 69.89 ± 5.90 kg ; 170.21 ± 5.31 cm, 81.98 ± 3.73 mmHg and 78.89 ± 3.71 mmHg respectively of healthy control subjects (Table 1).

Table 1. *General characteristics of PCOS patients and healthy control subjects.*

Anthropometric assays	Normal healthy control subjects (n=35)	PCOS patients (n=35)
Subject Number	35	35
Gender (Male/ Female)	18/17	20/15
Height (cm)	170.21 ± 5.31	172.02 ± 5.21 (+1.06)
Weight (kg)	69.89 ± 5.90	83.48 ± 5.11 (+19.44)
Age (years)	42.12 ± 5.72	43.99 ± 4.49 (+4.43)
Blood pressure systolic (mmHg)	81.98 ± 3.73	120.27 ± 3.19 (+46.70)
Blood pressure diastolic (mmHg)	78.89 ± 3.71	98.99 ± 3.78 (+25.47)

Glucose and HbA1c levels: A nominal increase was observed in fasting glucose and HbA1C levels by 17.80%

(from 80.92± 4.75 to 195.32 ± 7.08 mg/dL) and 1.59% (from 5.67 ± 0.34 to 5.76 ± 0.29 g%) respectively (Table 2).

Table 2. *Alterations in plasma fasting blood glucose and blood HbA1 C levels in PCOS patients and healthy control subjects.*

Biochemical assays	Normal healthy control subjects (n=35)	PCOS patients (n=35)
Fasting Blood Glucose (mg/dL)	80.92 ± 4.75[a]	95.32 ± 7.08 (+17.80)[b NS]
HbA1C (g%)	5.67 ± 0.34[a]	5.76 ± 0.29 (+1.59)[b NS]

a-Values expressed as Mean ± SD of 150 observations.
b-Values in parentheses representing the percentage change w. r. t healthy control subjects.
NS= Not Significant

Serum trace elements: The status of trace elements such as Ferritin, Magnesium, Copper and Calcium in the serum of PCOS patients and healthy control subjects are summarized in the Table 3. The serum Ferritin and Copper was 202.91 ± 13.18 ng/ml and 99.99 µg/dL respectively in PCOS patients and 110.19 ± 8.14 ng//ml and 99.99 µg/dL µg/dL respectively in healthy control subjects was observed. A significant increase in the levels of serum ferritin and copper by 84.14% ($P \leq 0.001$.) and 35.33% ($P \leq 0.05$) respectively was recorded in PCOS patients in comparison to healthy subjects (Table 3) while a significant fall in zinc ($P \leq 0.05$), magnesium ($P \leq 0.05$) and selenium ($P \leq 0.05$) was found in PCOS patients respectively in PCOS patients with respect to normal healthy control subjects (Table 3).

Table 3. *Alterations in serum Ferritin, Zinc, Copper, Magnesium and Selenium in PCOS patients and healthy control subjects.*

Biochemical Assays	Healthy Control Subjects (n=35)	PCOS Patients (n=35)
Ferritin (ng/ml)	110.19 ± 8.14	202.91 ± 13.18(+84.14)**
Zinc (µg/dL)	91.89 ± 6.23	56.61± 4.59 (-38.39)
Copper (µg/dL)	99.99 ± 5.32	135.32±8.52 (+2.05)NS
Magnesium (mEq/ dL)	19.11± 1.88	11.97 ± 0.22 (-37.36)
Selenium (ng/ml)	61.74 ± 2.36	29.15 ± 1.56 (-52.78)*

NS= Not Significant; *(P<0.01) Significant; **(P<0.001) Highly Significant

Discussion

Ferritin

A significant ($P \leq 0.01$) increase in serum ferritin, a reflector of body iron stores was observed in PCOS patients by 88.92% (from 107.92 ± 9.34 ng/ml to 203.89 ± 15.38 ng/ml)(Table 3). A significant increase in iron indices, especially ferritin indicates a high availability of iron in different tissues of patients with PCOS [13] Increases in iron store and ferritin levels in PCOS may be attributed to the absence of regular menstrual blood loss, leading to iron overload, as serum ferritin levels have been observed to be higher in oligoamenorrheic patients compared with regularly menstruating women. Oxidative stress induces ferritin synthesis to reduce further oxidative damage, given that ferritin neutralizes highly toxic unbound iron. Previous literature reports [13-15]. Research has also shown that oxidative stress may be increased in women with PCOS. Hyperinsulinemia may also account for this phenomenon. Insulin resistance, which is prevalent in patients with PCOS, may likewise contribute to increased serum ferritin and body iron stores, because insulin may stimulate intestinal iron absorption by up regulating the activity of hypoxia-inducible factor-1α and down regulating hepcidin expression [13,15]. Moreover, hyperandrogenemia, which affects erythropoiesis, is widely known as a critical component of PCOS.

Copper (Cu)

A significant increase in Cu concentration by 35.33% from 99.99 ± 5.32 to 135.32 ± 8.52 µg/Dl (Table 3) was observed in PCOS patients in comparison to healthy control subjects (Table 3). Cu, an essential trace element in the human body for the production of energy and required as a cofactor for many enzymes in critical metabolic pathways including cytochrome oxidase, superoxide dismutase, ascorbic acid oxidase, and tyrosinase. Recently, it has been reported that Cu interacts with key neuropeptides in the hypothalamic-pituitary-gonadal axis, notably, Gonadotropin-Releasing Hormone (GnRH) and neurokinin B, and promotes anovulatory menstruation [16]. Excessive levels of Cu induce oxidative stress *via* Fenton and redox reactions, resulting in increased production of Reactive Oxygen Species (ROS), a hallmark for the pathophysiology of many non-communicable diseases[17,18] and can alter the steroid genesis process in the ovary, leading to increased androgen levels, disturbance in follicular development, and infertility. Moreover, insulin resistance is reported to be linked with oxidative stress, which may mediate PCOS occurrence through facilitating secretion of excessive levels of androgens from ovaries and adrenal glands [18].

Zinc (Zn)

Zn acts as a stabilizer and cofactor for many enzymes and is an essential element for hormonal function was decreased significantly ($P \leq 0.05$) by 49.27% (from 91.89 ± 6.23 µg/dL to 46.61 ± 4.59 µg/dL) in PCOS patients in comparison to healthy control subjects (Table 3).In the state of physiochemically, Zn is able to act to act as antioxidant by protecting the sulfhydryl groups of different proteins and enzymes against free radicals and inhibits the oxidative stress. Zn is one of the trace elements crucial for normal insulin hormone response

especially in the downstream insulin signaling and it is essential for normal insulin hormone synthesis, storage and release as well as it may be has an insulin like activity upon binding to insulin receptor [17,19]. A significant higher level of Cu in PCOS patients may directly affect infertility rate by lowering progesterone levels resulting in anovulation, implantation failure or luteal phase deficit. It is also uncertain that whether high levels of Cu are related to hidden inflammatory conditions or not? Because ceruloplasmin, acute phase protein may have the ability to increase in the serum Cu and decrease Zn levels [19]. This imbalance between the levels of Cu and Zn could be the major cause of infertility. The excessive amounts of Cu may interfere with neuronal signaling in central nerve system, which are good responsible for the neuroendocrine regulation of fertility.

Magnesium (Mg)

Mg has been identified as a necessary nutrient for energy production and synthesis of nucleic acids was decrease by 52.32% (from 25.11 ± 2.88 mEq/dL to 0.97 ± 0.221 mEq/dL) in PCOS patients w. r. t. healthy control subjects. Decreased levels of Mg have been shown in various reproductive events like infertility, spontaneous abortions, congenital anomalies, preeclampsia, placental abruption, premature rupture of membranes, still births and low birth weight [20]. Furthermore, decreased level of Mg can paradoxically increase the risk factor of oncogenesis. It has been reported that Mg is central in the cell cycle, and that its deficiency is an important conditioner in precancerous cell transformation.

Selenium (Se)

A significant decrease ($P<0.05$) by 52.78% (from 61.74 ± 2.36 ng/ml to 29.15 ± 1.56 ng/ml) was recorded in Se level in PCOS patients w. r. t. healthy control subjects (Table 3). A significant ($P<0.05$) decrease in Se levels in PCOS patients might be due to expose the women to oxidative stress, which is a responsible for the pathophysiology of many diseases [17,18]. A significant fall in Se levels in PCOS patients might give an indication of increased free radical production or highly scavenging activity of either selenium or glutathione peroxidase enzyme might fall in PCOS women's. Also, there are some scientific reports have demonstrated that selenium plays a significant vital role in the undisturbed functioning of the reproductive system which prove to the correlations between the Se intake and the fertility as well as disorders of procreation processes [12]. In the present study, a significant decrease in Se levels in PCOS patients are agreement with the literature reports.

Conclusion

All the for mentioned observations of overload of essential trace elements such as ferritin, Cu while fall in Se, Zn and Mg levels are an important etiological role in the pathogenicity and increased complication of PCOS. The therapeutic interventions with these trace elements as supplement in a suitable formula may be beneficial to improve the conditions of PCOS patients. The therapeutic interventions with these trace elements as supplement in a suitable formula might be beneficial to improve the conditions of PCOS patients.

Acknowledgment

I thank to the faculty of Gynecology and Obstetrics Department, Government Medical College and Rajindra Hospital, Patiala for providing patients and their cooperation. My thanks to participants for giving us the opportunity for being the part of this study. I would like to thank Multi-Disciplinary Research Unit (MRU), Government Medical College and Rajindra Hospital-Patiala for providing Equipments/Machinery to conduct this research work.

Conflict of Interest

None

References

1. Setji TL, Brown AJ. Polycystic ovary syndrome: Update on diagnosis and treatment. Amer J Med 2014; 127: 912-919.
2. Hayes MG, Urbanek M, Ehrmann DA, Armstrong LL, Lee JY, Sisk R. Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. Nature Comm 2015; 6.
3. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and longterm health risks related to Polycystic Ovary Syndrome (PCOS). Hum Reprod 2004; 19: 41-47.
4. Gonzalez F, Rote NS, Minium J, Kirwan JP. Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab 2006; 91: 336-340.
5. Papp LV, Lu J, Holmgren A, Khanna KK. From selenium to selenoproteins: Synthesis, identity, and their role in human health. Antioxid Redox Signal 2007; 9: 775-806.
6. Lippman SM, Goodman PJ, Klein EA, Parnes HL, Thompson IM Jr, Kristal AR, Santella RM. Designing the selenium and vitamin E cancer prevention trial (SELECT). J Natl Cancer Inst 2005; 97: 94-102.
7. Kauffman RP, Tullar PE, Nipp RD, Castracane VD. Serum magnesium concentrations and metabolic variables in polycystic ovary syndrome. Acta Obstet Gynecol Scand 2011; 90: 452-458.
8. Milal MAJ. Some altered trace elements in patients with polycystic ovary syndrome. British J Med & Med Res 2017; 20: 1-10.
9. Barbagallo M, Dominguez LJ, Galioto A, Ferlisi A, Cani C, Malfa L, Pineo A, Busardo' A, Paolisso G. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Mol Aspects Med 2003; 24: 39-52.
10. Mamza, YP, Abdullahi ZB, Gali RM, Mshelia DS, Genesis RY, Habu SA. Status of serum Zinc and magnesium among Type 2 diabetic subjects in maiduguri. IOSR J Dental and Medi Sci 2016; 15: 66-70.

11. Jamilian M, Foroozanfard F, Bahmani F, Talaee R, Monavari M, Asemi Z. Effects of zinc supplementation on endocrine outcomes in women with polycystic ovary syndrome: A randomized, double-blind, placebo-controlled trial. Biol Trace Elem Res 2016; 170: 271-278.
12. Pieczyńska J, Grajeta H. The role of selenium in human conception and pregnancy. J Trace Elem Med Biol. 2015; 29: 31-38.
13. Luque-Ramírez M, Álvarez-Blasco F, Botella-Carretero J, Sanchón R, San Millán J, Escobar-Morreale HF. Increased body iron stores of obese women with polycystic ovary syndrome are a consequence of insulin resistance and hyperinsulinism and are not a result of reduced menstrual losses. Diabetes Care 2007; 30: 2309-2313.
14. Valkenburg O, Steegers-Theunissen RP, Smedts HP, Dallinga-Thie GM, Fauser BC, Westerveld EH. A more atherogenic serum lipoprotein profile is present in women with polycystic ovary syndrome: A case-control study. J Clin Endocrinol Metab 2008; 93: 470-476.
15. Botella-Carretero JI, Luque-Ramirez M, Alvarez-Blasco F, San Millan JL, Escobar-Morreale HF. Mutations in the hereditary hemochromatosis gene are not associated with the increased body iron stores observed in overweight and obese women with polycystic ovary syndrome (Letter). Diabetes Care. 2006; 29: 2556.
16. Peacey L, Elphick MR, Jones CE. Roles of copper in neurokinin B and gonadotropin-releasing hormone structure and function and the endocrinology of reproduction. Gen Comp Endocrinol. 2020; 287: 113342.
17. Sak S, Uyanikoglu H, Incebiyik A, Incebiyik H, Hilali NG, Sabuncu T. Associations of serum fetuin-A and oxidative stress parameters with polycystic ovary syndrome. Clin Exp Reprod Med. 2018; 45: 116-121.
18. Sulaiman MA, Al-Farsi YM, Al-Khaduri MM, Saleh J, Waly MI. Polycysticovarian syndrome is linked to increased oxidative stress in omani women. Int. J. Womens Health 2018; 10: 763-771.
19. Hussien, KA, Al-Salih, RM, Ali SA. Evaluation of hormones and trace elements in women with unexplained infertility. Thi Qar University 2017; 14: 94-108.
20. Chen P, Totten M, Zhang Z, Bucinca H, Erikson K, Santamaría A, Bowman AB. Aschner M. Iron and manganese-related CNS toxicity: Mechanisms, diagnosis and treatment. Exp Rev Neurother 2019; 19: 243-260.

***Correspondence to:**

Kuldip Singh

Department of Biochemistry

Government Medical College-Patiala

Punjab

India