ISSN (Print) 0975-685X
ISSN (Online) 0975-864X

J. Appl. Biosci., 50(1): 53-57, January-June, 2024

COMPARATIVE ANALYSIS OF TRACE ELEMENT LEVELS IN DISORDERS OF SEX DEVELOPMENT CASE AND CONTROL GROUPS: A DETAILED OBSERVATIONAL STUDY

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ABSTRACT

Disorders of Sex Development (DSDs) refer to a group of congenital conditions characterized by atypical development of chromosomal, gonadal, or anatomical sex. This can result in variations in genitalia that do not fit typical definitions of male or female. This study examines trace element levels in patients with DSDs using Atomic Absorption Spectroscopy (AAS). The analysis focused on iron (Fe), copper (Cu), zinc (Zn), chromium (Cr), selenium (Se), and magnesium (Mg). The concentrations of these elements were compared between a group of DSD patients (case group) and a control group without DSDs. The results indicated a statistically significant difference in iron levels, with the DSD patients showing notably higher concentrations ($P < 0.05$) compared to the control group. In contrast, the levels of copper, zinc, chromium, selenium, and magnesium did not show significant differences between the two groups. The elevated iron levels in DSD patients suggest a potential alteration in iron metabolism associated with these disorders. This finding highlights the importance of further research to understand the role of iron in the etiology and management of DSDs. The lack of significant differences in the other trace elements, such as Cu, Zn, Cr, Se, and Mg, suggests that these elements might not be as critically involved in the pathophysiology of DSDs as iron. Nonetheless, the study underscores the necessity for a deeper exploration of trace element imbalances and their potential impacts on health outcomes in DSD patients. Further investigations could provide insights into whether these imbalances contribute to the development or progression of DSDs and how they might be addressed in clinical practice.

KEY WORDS: Congenital illnesses, ambiguous genitalia, Disorder Sex Development, trace elements

INTRODUCTION

A class of congenital illnesses known as Disorders of Sex Development (DSDs) are characterized by aberrant chromosomal, gonadal, or anatomical sex development (Mendonca *et al.,* 2009). These abnormalities can manifest as a range of phenotypes, such as ambiguous genitalia (Dreger *et al.,* 2020) and inconsistencies between chromosomal and phenotypic sex, posing major health and emotional issues for afflicted individuals and families (Sax *et al*., 2002). The incidence of DSDs is estimated to be 1 in 4,500 to 1 in 5,500 live births, indicating the complex and varied nature of the illnesses (Lee *et al*., 2006). A complicated interaction between genetic, hormonal, and environmental variables results in DSDs (Mendonca *et al*., 2009). Additional understanding of the mechanisms behind these disorders has been made possible by recent advancements in genetic and molecular research, which has led to identification that is more accurate and customised treatment plans (Mouriquand *et al*., 2016; Mett & Müller *et al.,* 2021). Similarly, there is a growing emphasis on patient-centred care, prioritising informed consent, interdisciplinary assistance, and respect for the individual's gender identity (Karkazis *et al*., 2008). Trace elements, essential micronutrients required in small

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amounts for various physiological processes, have been increasingly recognized for their role in human health and disease (Diplock *et al*., 1987). Trace elements, including iron, zinc, copper, selenium, and manganese, are vital micronutrients required for various physiological processes (Prashanth *et al.,* 2015). They act as cofactors for numerous enzymes, contribute to antioxidant defence, and are intricate in regulating gene expression (House *et al.,* 1999). For instance, zinc is crucial for properly working the reproductive system, influencing hormone production and the activity of sex steroid receptors (Stefanidou *et al*., 2006). Copper and selenium are integral to antioxidant enzymes, protecting developing tissues from oxidative damage (Diplock *et al*., 1987), which is particularly crucial during the rapid cell division and differentiation that occur in embryogenesis (House *et al.*, 1999). Among these, iron plays a critical role in numerous biological functions, including oxygen transport, DNA synthesis, and cellular respiration (Beard *et al.*, 2001). Its role in sex development, however, is a relatively unexplored area of research that warrants further investigation (Cai *et al*., 2020).

In addition to significantly impacting general health, iron dysregulation or deficiency may also impact the development and function of the reproductive system (Ladjouze & Donaldson *et al.*, 2019). Iron is required for producing haemoglobin and myoglobin (Sayyad *et al.,* 2012), two proteins required for oxygen delivery and storage. Furthermore, the synthesis of steroid hormones which are essential for healthy sexual development–is carried out by iron-dependent enzymes (Beard *et al.* 2001). Since abnormal hormone levels are a prevalent hallmark of DSDs, the interaction between iron metabolism and hormonal control is very important. Studies have indicated that iron deficiency during critical periods of development can lead to alterations in sex hormone levels, potentially impacting gonadal development and function (House *et al.*, 1999). Conversely, iron overload conditions, such as hemochromatosis, can also disrupt endocrine function and reproductive health (McLaren & Gordeuk*et al.*, 2009). Thus, maintaining optimal iron levels is crucial for normal sexual development and reproductive health.

The purpose of this research is to determine the trace element content of DSDs, emphasising the function of iron as a trace element in sexual development. Using Atomic Absorption Spectroscopy (AAS) to investigate the biological roles of trace elements and their effects on endocrine health, this study aims to clarify the pathways which iron could involve dysregulation in the pathophysiology of DSDs. By using an integrative approach, clinical outcomes for people with DSDs may be improved in the long run by creating new opportunities for research and therapy approaches.

MATERIALS AND METHODS

The prospective study was carried out with ethics committee approval at Banaras Hindu University's Department of Anatomy, Institute of Medical Sciences. Before participation, every participant gave written, informed consent. The study enrolled a total of 16 patients identified with Disorders of Sex Development (DSDs) with penile scrotal hypospadias, definite as congenital conditions wherein the growth of chromosomal, gonadal, and anatomic sex is unusual. Phenotypic sex determination depends on the type of gonad that develops in the embryo, a process influenced by genetic inheritance. Gonadal development is unique, as gonads have the potential to differentiate into either testes or ovaries (Sax *et al.*, 2002). All patients underwent examination within the first five days of onset of DSD symptoms. The study included patients aged between 6 and 14 years and excluded those aged 18 years and older. The control group comprised 11 asymptomatic healthy volunteers, both female and male, with normal physical examinations and unremarkable medical histories.

Blood samples:

A Blood samples were collected from the Department of Pediatrics, Sir Sundar Lal Hospital, Institute of Medical Sciences, Banaras Hindu University. A total of 4 ml of blood was drawn from each participant in case and control groups and transferred into biochemical tubes. The samples underwent centrifugation for 7 minutes at 3000 rpm using the Remi R-8c Laboratory Centrifuge. After centrifugation, the serum was carefully separated and stored at -80°C until processing. Before processing, the samples were stored at -20°C and subsequently shifted to a refrigerator set at 4°C on the processing day. The AAS (Atomic Absorption Spectroscopy) procedure comprised four distinct steps, outlined as follows:

Diluent Preparation:

The method involves combining specific quantities of 1% Nitric acid and 0.4% Triton X with distilled water to create the diluent solution.

Measure 500 µl of 1% Nitric acid and 200 µl of 0.4% Triton X accurately. Place these measured quantities of Nitric acid and Triton X into a 50 ml falcon tube. Add 49.3 ml of distilled water to the falcon tube containing Nitric acid and Triton X. Mix the contents thoroughly to ensure homogeneity. The resulting solution serves as the prepared diluent.

Standard preparation:

The standard preparation procedure involves two different volumes for achieving different concentrations.

For a 50 ml standard preparation with a concentration of 1 ppm, 50 µl of stock is dissolved in 49.95 ml of 1% nitric acid. Conversely, for a 10 ml standard preparation at a concentration of 20 ppb, 0.2 ml of stock is dissolved in 9.8 ml of 1% nitric acid.

Sample preparation

Regarding sample preparation, 800 il of diluent is mixed with 200 il of the sample in a 1 ml sampler tube.

Blank preparation:

For blank preparation, 300 µl of 1% nitric acid was combined with 800 µl of diluent in a sampler tube. These preparations ensure accurate calibration and baseline measurements necessary for the AAS analysis. Serum levels of trace elements (Fe, Zn, Cu, Co, Mg, Sn, and Cr) were determined by the Perkin Elmer Atomic Absorption Spectrophotometer: Pinnacle 900T Graphite Furnace method (Mokgohloa *et al.,* 2022) in the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University. The absorption wavelengths of Fe (248.3 nm), Zn (213.9 nm), and Cu (324.8 nm) are distinct for each element. Through AAS analysis, these particular wavelengths make it easier to detect and quantify the constituents in the sample.

Statical Analysis:

Statistical analysis was conducted using GraphPad Prism 9 software (GraphPad Software Inc., San Diego, USA (Belayneh *et al.,* 2022). Calculating the mean, median, standard deviation, coefficient of variation, standard error, and minimum-maximum values was part of the descriptive analysis process. The normal distribution of trace elements was assessed using the Kolmogorov-Smirnov test. If the normal distribution was confirmed, an unpaired parametric two-tailed t-test was employed; otherwise, the Mann-Whitney test was utilized. Outliers were identified using the Tukey method with Violine Graph plotting and were subsequently excluded from the analysis. Statistical significance was established at $P < 0.05$ (*).

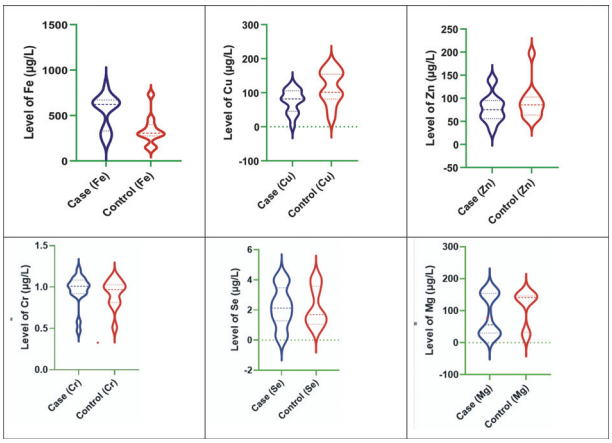

Fig. 1. Showing Violine Graph of Trace elements of case and control groups

Table 1: Comparison of the trace elements in the case and control groups

	Case Group		Control Group		
Variables	M(N=16)	SD	M(N=11)	SD	P
Fe (mg/dl)	545.9	188.5	337.2	160.3	**
Cu(mg/dl)	74.94	34.08	115.5	50.00	ns
Zn(mg/dl)	78.30	30.57	91.90	39.66	ns
Cr(mg/dl)	0.9604	0.1889	0.9202	0.1744	ns
Se(mg/dl)	2.202	1.340	2.195	1.283	ns
Mg(mg/dl)	87.33	63.71	112.0	54.90	ns

NOTE: M- Mean, SD- Standard Deviation, Fe= Iron, Cu= Copper, Zn=Zinc, Cr=Chromium, Se=Selenium, Mg=Magnesium

RESULT

Measurements and comparisons of the amounts of different trace elements were made between the case and control groups. as shown in (Fig. 1). The mean (M) and standard deviation (SD) for each trace element are presented in Table 1. Statistical analysis was conducted to determine the significance of differences between the groups.

Iron (Fe)

The mean iron level in the case group was significantly higher (M = 545.9 mg/dl, SD = 188.5) compared to the control group (M = 337.2 mg/dl, SD = 160.3). This difference was statistically significant ($t(27) = P < 0.01$), indicating a higher concentration of iron in the case group.

Copper (Cu)

The mean copper level in the case group was 74.94 mg/dl (SD = 34.08), while in the control group, it was 115.5 mg/dl (SD = 50.00). The difference between these means was not statistically significant (ns), suggesting no significant difference in copper levels between the two groups.

Zinc (Zn)

The mean zinc level in the case group was 78.30 mg/dl (SD = 30.57), compared to 91.90 mg/dl (SD = 39.66) in the

control group. This difference was not statistically significant (ns), indicating similar zinc levels in both groups.

Chromium (Cr)

Chromium levels were slightly higher in the case group (M = 0.9604 mg/dl, SD = 0.1889) compared to the control group (M = 0.9202 mg/dl, SD = 0.1744), but this difference was not statistically significant (ns).

Selenium (Se)

The selenium levels were very similar between the case group (M = 2.202 mg/dl, SD = 1.340) and the control group (M = 2.195 mg/dl, SD = 1.283), with no statistically significant difference (ns).

Magnesium (Mg)

The mean magnesium level in the case group was 87.33 mg/dl (SD = 63.71), whereas it was 112.0 mg/dl (SD = 54.90) in the control group. This difference was not statistically significant (ns), indicating no significant difference in magnesium levels between the groups.

Overall, these results indicate that, among the trace elements measured, only iron showed a significant difference between the case and control groups, with higher levels observed in the case group. Other trace elements, including copper, zinc, chromium, selenium, and magnesium, did not show significant differences between the groups. This study aims to clarify the role of iron dysregulation could be involved in the pathophysiology of DSDs.

DISCUSSION

The present study aimed to compare the levels of various trace elements between a case group and a control group to explore potential differences that could contribute to the pathophysiology of Disorders of Sex Development (DSDs). The findings revealed a significant elevation in iron (Fe) levels in the case group compared to the control group, while the levels of copper (Cu), zinc (Zn), chromium (Cr), selenium (Se), and magnesium (Mg) did not differ significantly between the two groups.

Iron (Fe) Dysregulation

The significant increase in iron levels in the case group (M = 545.9 mg/dl, SD = 188.5) compared to the control group (M = 337.2 mg/dl, SD = 160.3) suggests that iron dysregulation might play a crucial role in the pathophysiology of DSDs. Elevated iron levels have been associated with oxidative stress and tissue damage, which could contribute to abnormal development and function in individuals with DSDs. Iron is a critical component of haemoglobin and various enzymes, and its imbalance can disrupt numerous physiological processes. The significant difference observed in our study underscores the need for further research to investigate how iron dysregulation impacts DSDs and explore potential therapeutic interventions aimed at normalizing iron levels.

Copper (Cu) Levels

The copper levels in the case group (M = 74.94 mg/dl, SD = 34.08) were lower than in the control group (M = 115.5 mg/dl, SD = 50.00), although this difference was not statistically significant. Copper is vital for various enzymatic reactions and the functioning of the nervous and immune systems. Despite the lack of a significant difference, the observed trend of lower copper levels in the case group warrants further investigation, as even non-significant trends might have biological relevance in the context of DSDs.

Zinc (Zn) Levels

Zinc levels did not show a significant difference between the case (M = 78.30 mg/dl, SD = 30.57) and control (M = 91.90 mg/dl, SD = 39.66) groups. Zinc is essential for DNA synthesis, cell division, and protein synthesis. The similar levels of zinc between the groups suggest that zinc deficiency or excess is not a contributing factor to DSDs in this study population. However, zinc's role in reproductive health and development means that its status should continue to be monitored in future studies.

Chromium (Cr), Selenium (Se), and Magnesium (Mg) Levels

The levels of chromium, selenium, and magnesium also did not differ significantly between the case and control groups. Chromium plays a role in glucose metabolism, selenium is important for antioxidant defense and thyroid function, and magnesium is involved in numerous biochemical reactions. The lack of significant differences in these trace elements suggests that their dysregulation may not be directly involved in the pathophysiology of DSDs, at least in the population studied. However, maintaining adequate levels of these elements is crucial for overall health, and their potential indirect effects on DSDs should not be overlooked.

CONCLUSION

In conclusion, our study highlights a significant difference in iron levels between individuals with DSDs and controls, suggesting that iron dysregulation may be a key factor in the pathophysiology of these conditions. The lack of significant differences in the other trace elements measured indicates that their roles may be less direct or pronounced. These findings contribute to a better understanding of the biochemical landscape in DSDs and

underscore the importance of further research into the mechanisms and clinical implications of iron dysregulation in these disorders. Future studies should focus on elucidating the pathways through which iron affects DSD development and exploring targeted interventions to correct iron imbalances.

Conflict of interest: None

Informed consent : Yes, taken from the Patient.

ACKNOWLEDGMENTS

We are sincerely gratitude to the Multi-Disciplinary Research Units (MRUs) Laboratory, a grant by ICMR Department of Health Research and also thanking to the participants, without their permission and consent, this work would not be possible.

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