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RESEARCH ARTICLE

Analysis and Comparison of Zinc Micro Element Concentration Present in Human Seminal Plasma of Asthenospermia and Normospermia Conditions Using Atomic Absorption Spectroscopy for Male Infertility Diagnosis Related to Motility Issues

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ABSTRACT

Aim: The major aim of this study is to compare Zinc (Zn) concentration present in human seminal plasma of asthenospermia (motility issues) and normospermia semen samples for identification of clinical relevance. **Materials and Methods:** Semen samples of normospermia (N=75), asthenospermia (N=75) were collected and semen analysis report has been done by standard world health organization protocol. Zn concentration was evaluated by using atomic absorption spectroscopy (AAS) for both the groups by using standard protocol. **Results:** Independent sample T-test on fertile and infertile men (asthenospermia and normospermia group) reveals that the zinc concentration is statistically insignificant (P=0.367). Zinc concentration was found to be high in normospermia (mean 2.5 mg/ml) over asthenospermia (mean 0.90 mg/ml) samples. **Conclusion:** Zn being an essential element for normal functioning for fertilization, its absence or less concentration may lead to infertility. We found high concentration of Zn in normospermia which reflects normal conditions for fertilization and less concentration in asthenospermia reflecting infertile conditions.

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Introduction

This research is about clinical diagnosis of human male infertility for asthenospermia (motility issues) and normospermia conditions. This research is important as infertility affects four out of ten couples worldwide (Vaghela et al. 2016) in which male infertility accounts for half of all cases. As a consequence, a proper assessment of human male infertility is required. Several organic and inorganic elements found in human seminal plasma play important roles in sperm capacitation (Mirnamniha et al. 2019), metabolism, and acrosome reaction.

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12 research articles were published in IEEE explore and 859 research articles were published in science direct. In this work the focus is on two types of Solar cells, Glass (protective layer) and ZnO (protective layer) based solar cell. Shruti Sharma et.al, explained that solar cells are the renewable source devices used for converting light energy to electrical energy based on the concept of photovoltaic effect. Mehreen Gul et.al, explained photovoltaic effect as the generation of a potential when radiation ionises the area or near the built-in theoretical semiconductor firewall(Gul, Kotak, and Muneer 2016) and are a great alternative for fossil fuels and petroleum deposits(S. Sharma, Jain, and Sharma 2015). Xinrui Wang et.al, has done research on implementation of

photovoltaic power generation along with hydro power generation systems by conservation of energy in storage systems and proposed that encapsulation technology coupled with silicon helps to produce solar cells with higher stability(D. Wang et al. 2016). S.Gall et.al proposed ZnO-Al

This study results may lead to proper diagnosis of motility issues related male infertility. This study results can be applied in the area of andrology and in assisted reproductive technology (ART) centers for diagnosis of human male infertility.

Literature suggests zinc is an essential micronutrient found in humans and is linked to a variety of physiological functions especially in fertilization. Highly cited article reveals the importance of zinc for sperm motility and its evaluation is important for proper diagnosis of male infertility and shows Zn in high concentration (2.75 mg/ml) (Erdem et al. 2013). Zn content in human semen was 85 to 90 times higher in seminal plasma than that of blood. Zinc acts as a shield for sperm cells while they enter the female reproductive tract (Mohammad-Hasani, Colagar, and Fallah 2019), protecting them from chromosomal damage (Kovacik et al. 2018; S. Vickram et al. 2021). Zinc supplementation improves semen parameters such as volume, forwarded motility, and normal morphology (Zhao et al. 2016). Zinc ions in seminal fluid for normospermia conditions (2.75 mg/ml) correlates positively with all semen parameters was the best study we analyzed in this area (Robertson and Sharkey 2016). Zinc deficiency in seminal plasma causes low sperm counts and poor sperm production (Wang et al. 2015).

Previously our team has a rich experience in working on various research projects across multiple disciplines (Sathish and Karthick 2020; Varghese, Ramesh, and Veeraiyan 2019; S. R. Samuel, Acharya, and Rao 2020; Venu, Raju, and Subramani 2019; M. S. Samuel et al. 2019; Venu, Subramani, and Raju 2019; Mehta et al. 2019; Sharma et al. 2019; Malli Sureshbabu et al. 2019; Krishnaswamy et al. 2020; Muthukrishnan et al. 2020; Gheena and Ezhilarasan 2019; Vignesh et al. 2019; Ke et al. 2019; Vijayakumar Jain et al. 2019; Jose, Ajitha, and Subbaiyan 2020). Now the growing trend in this area motivated us to pursue this project.

There is no clinical conclusive study for Zn concentration and its impact on human male infertility, especially with asthenospermia (motility issues). Authors were already expertised in this field of research on human male infertility and semen sample handling. The aim of the current study is to analyse and compare the Zinc microelement concentration present in human seminal plasma of asthenospermia and normospermia conditions using AAS. Normospermia refers to the conditions where males show normal semen parameter values whereas asthenospermia refers to the condition where males show a low level of motile sperms (less than 20 %) leading to male infertility (World Health Organization 2010).

Materials & Methods

This study was conducted at biochemistry lab in Saveetha school of engineering. Samples were collected in accordance with the World Health Organization (WHO) standard procedure. Patient's consent was collected before performing research. Semen was collected in plastic vials and was observed to determine several parameters. Sample size was calculated by using previous study results (A. S. Vickram et al. 2013), in Clincalc.com by keeping threshold 0.05, G power 80%, confidence interval 95 % and enrollment ratio as 1.

Two different groups were taken for the analysis one is normospermia group (fertile men N=75) and the other one is asthenospermia which is an infertile condition (N=75). Computer assisted semen analysis (CASA) -German made, MTA 50 CSA and AAS- KRUSS optronic GmbH, and flame photometry-labtronics flame photometer model LT-65, were used in this study for various analysis. Semen analysis was done by CASA (A. S. Vickram et al. 2020) and then centrifugation is done at 5000 RPM for 10 mins and 10000 RPM for 15 mins which separates the seminal plasma (A. S. Vickram et al. 2013) and this was further checked for quantification of zinc concentration (innovative approach for diagnosis of male infertility) detected by using AAS.

Semen collected in a plastic container was analysed for different semen parameters such as pH, volume, sperm concentration, rapid motility. Zinc concentration analysis was done using AAS and calibration was done which evaluates the concentration of zinc in the samples. The data was collected as volume (ml), pH, sperm motility (%), sperm morphology (%), sperm concentration (millions/ml) and zinc concentration (mg/ml).

Statistical Analysis

Statistical comparison of zinc concentration between fertile and infertile groups was done through SPSS version 21. There are no dependent variables and the independent variables are volume, motility, morphology and zinc concentration. Analysis was done for mean, standard deviation, independent T test.

Results

Semen analysis was performed and reported in Table 1 for major parameters like volume, pH, sperm concentration, total motility, rapid progressive motility, and normal morphology for both normospermia and asthenospermia conditions. Drastic reduction was found in motility in asthenospermia condition (mean 8.0 %) when compared with normospermia (mean motility 53.2%).

Table 1. Comparison of semen parameter values for asthenospermia and normospermia conditions for major semen parameters (Mean and Standard error of mean). All the values were in accordance with WHO standard values in normospermia and not in the case of the asthenospermia group. Sperm motility seems to be higher in normospermia (mean 53.2 %) and significantly low in asthenospermia (mean 8 %).

Semen Category	Volume (ml)	pH	Sperm Conc. (in Millions/ ml)	Total Motility (%)	Rapid Progressive Motility	Normal Morphology (%)
Normospermia (N=75)	3.4±0.7	7.4±0.1	71.3±8.0	53.2±6.1	20.0±1.3	30.5±2.6
Asthenospermia (N=75)	3.0±0.1	7.2±0.2	22.2±3.0	8.0±1.7	5.4±0.0	17.7±3.5

Zn concentration was evaluated for both the conditions and depicted in Table 2. The data shows drastic reduction of Zn concentration in asthenospermia (mean 0.90 mg/ml) when compared to normospermia (mean 2.5 mg/ml).

Table 2. Comparison of zinc concentration (Mean and Standard error of mean) for normospermia and asthenospermia. Zn concentration seems to be higher in normospermia (2.5 mg/ml) over asthenospermia conditions (0.90 mg/ml)

Category	Zn Concentration(mg/ml)
Normospermia (N=75)	2.5±0.7
Asthenospermia (N=75)	0.90±0.57

The independent sample T test revealed that no statistical significant difference between these groups in Zn concentration evaluation, but there exists a drastic difference in the mean value for Zn concentration in both conditions (Table 3). The mean concentration of Zn in both conditions was compared in Fig. 1 and clearly reveals the low concentration of Zn in infertile condition (asthenospermia).

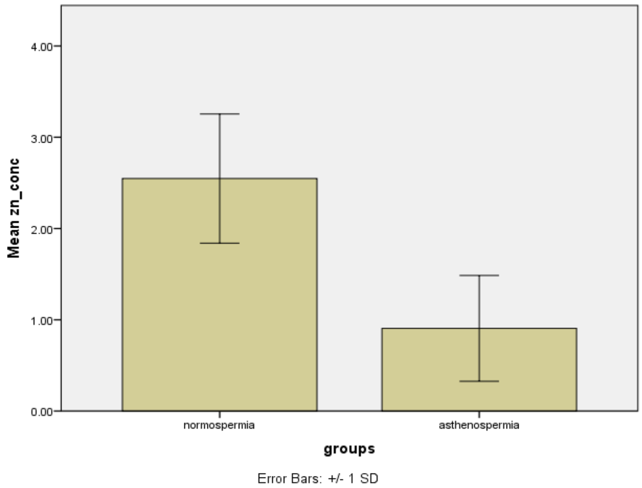

Figure 1. Bar chart representing the comparison of zinc concentration in normospermia and asthenospermia men (independent samples t test Means =/- 1 SD). It shows that the zinc concentration was less in asthenospermia men (0.90 mg/ml) and more in normospermia men (2.5 mg/ml). X axis: represents normospermia and asthenospermia conditions, Y axis: represents the mean value of Zn concentration ± 1 SD.

Table 3. Independent sample T test shows statistical insignificance (p=0.367) for Zn concentration between asthenospermia and normospermia groups.

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Diff	Std. Error Diff	95% Confidence Interval of the Difference	
									Lower	Upper
zn_conc	Equal variances assumed	.817	.367	15.54	148	.000	1.6417	.10562	1.4330	1.85046
	Equal variances not assumed			15.54	142.4	.000	1.6417	.10562	1.4329	1.85052

Discussion

In this study, we found Zn concentration was high in normospermia and low in case of asthenospermia. We also observed statistically insignificant (p=0.367) for Zn concentration between asthenospermia and normospermia done by using SPSS version 21. Similar kinds of results were obtained by Zhao et al (Zhao et al. 2016) in case of Zn concentration evaluation in the control group (fertile conditions).

Normospermia men have normal semen parameters whereas asthenospermia have less number of motile sperms and sperm quality and we got results in accordance with WHO, (World Health Organization 2010). Zn concentration in normospermia men (2.2mg/ml) is higher than the zinc concentration in asthenospermia men (0.6g/ml), which indicates the role of Zn trace element in sperm motility as Zn acts as an energy resource for sperm mobility (Harchegani et al. 2020). We could not find any opposing results for Zn concentration evaluation in normospermia and asthenospermia conditions.

Zinc level in seminal plasma of asthenospermia men is lower than normospermia men (Zhao et al. 2016). Changes in Zn concentration was related to sperm quality as they are involved in antioxidant balance and it also correlates with our results (Khan et al. 2011; Nenkova, Petrov, and Alexandrova 2017). Oxidative stress negatively affects quality of sperm in relevance with Zn concentration which reflects in our results in case of asthenospermia (Harchegani et al. 2020). Zinc plays a major role in male fertility (Khan et al. 2011). Zn helps in protection of sperm cells from oxidative damage and helps in maintaining sperm quality (Colagar, Marzony, and Chaichi 2009). Supplementation of Zinc was found to show increased fertility in asthenospermia men (Hadwan, Almashhedy, and Alsalman 2012).

Our institution is passionate about high quality evidence based research and has excelled in various fields ((Vijayashree Priyadharsini 2019; Ezhilarasan, Apoorva, and Ashok Vardhan 2019; Ramesh et al. 2018; Mathew et al. 2020; Sridharan et al. 2019; Pc, Marimuthu, and Devadoss 2018; Ramadurai et al. 2019). We hope this study adds to this rich legacy.

AAS had high sensitivity during standard curve preparation and relatively large samples could not be analyzed at a time. CASA had limitations in ensuring identification of forward motility sperms. We got better results because of the calibration process done perfectly for both CASA and AAS instruments. During semen collection we collected through non-toxic plastic vials which enable us to give better resutls in the smen parameter report. We followed the storage of semen samples as per the procedure of world health organizations leads to better results after freeze thaw.

The results obtained from this article may lead andrologists to include Zn evaluation as a mandatory test for preparation of seminal analysis report for proper diagnosis of male infertility. For this, Zn binding sites on sperm cells have to be elucidated.

Conclusion

Zinc concentration in seminal plasma of normospermia (2.5 mg/ml) men seems to be higher than asthenospermia men. This could be also one of the reasons for male infertility with motility issues as Zn plays a major role in motility of sperm. Proper evaluation of zinc concentration in seminal plasma could help andrologists to diagnose the male infertile conditions properly.

Declarations

Conflict of Interests

No conflict of interests in this manuscript.

Authors Contributions

Author YT was involved in data collection, data analysis, manuscript writing. Author VAS was involved in conceptualization, data validation, and critical review of manuscript.

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