African Scientist Vol. 24, No. 1 March 31, 2023
Printed in Nigeria
1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

AFS2022043/24103

Seminal Fructose and Zinc Levels with Blood Cadmium and Zinc Levels in Bronze Foundry Workers in Benin City, South-South Nigeria

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(Received December 28, 2022; Accepted in revised form February 20, 2023)

ABSTRACT: Today, occupational exposure to harmful substances is common in many nations across the globe. This occupational exposure in many countries is highly unregulated. Using the bronze casting industry as a case study, there is a heightened interest in bronze artwork but its creators (bronze casters) haven't been given much thought when it comes to exposure to toxicants. Bronze casting can result in exposure to harmful material into the body and this can have an impact on reproductive health. The aim of this study was to determine the levels of seminal fructose and zinc and blood zinc (Zn) and cadmium (Cd) among bronze casters and environmentally exposed persons as control group in Benin City, South-South, Nigeria. In this comparative cross-sectional research, 50 consenting participants were included, including 35 foundry employees (Bronze casters) who were age-and sex-matched and 15 environmental control persons. Participant's socio demographic information and work-related behaviors were gathered via a questionnaire. Inductively coupled plasma- Mass spectrometry (ICP-MS) was used to determine Cd and zinc concentrations in while colorimetric method based on modified karvonen method was used to measure seminal fructose. Using α=0.05 and the student's T-test was used to compare the means. The study's findings indicate that bronze casters had blood Cd levels that was significantly higher (0.99±0.07 g/L) than those in the environmental control group (0.63± 0.03 g/L) (p=0.00; p> 0.05), a significant higher concentration of Serum Zn was seen in the control than foundry workers. No significant difference was observed in the seminal Zn, fructose, true corrected fructose, or corrected fructose concentration, sperm cell concentration, volume, motility, morphology in the two groups. According to the findings of this research, Cd is accumulated in the blood of foundry workers with a significant reduction in serum Zn. This may portray a future threat to reproductive health if nothing is done to regulate the exposure of the workers and the environment.

Keywords: Bronze casters, Semen, Zinc, Cadmium

Introduction

The foundry is a specialized industrial activity that pours molten metal into molds after melting metals in a particular furnace to create a finished product. Foundries are often classified based on the kind of metal they work with, for example, an iron foundry, a brass foundry, or a bronze foundry. Benin bronze was used to adorn the royal house of the oba of the Benin Kingdom and is considered as one of the distinctive characteristics and legacies of the ancient Benin kingdom.

The foundry process involves pouring molten metal into a mold made with sand to form a desired shape. The mold may contain a refractory core which determines the dimensions of any internal cavity or hollow. After cooling, the mold is subjected to a ‘shake out' procedure to release the casting and removes the core. It is then cleaned, and any metal fragment is removed. Although, many changes have occurred in the foundry industry yet

the basic processes and associated hazards have remained much the same in many foundries (Riberio *et al.*, 2006).

Semen is made of spermatozoa, which is diluted by seminal fluid secreted by seminal vesicles and the prostate with a subtle contributions from Cowper's glands and epididymis for the normal functioning of spermatozoa (Plant *et al.*, 2015).

Fructose is a important biomarker for seminal fluid investigation, it provides aerobic and anaerobic source of energy for sperm (Mann and Lutwak-Mann, 1981) and has been transversely linked with progressive sperm motility and viscosity (Fabiani , *et al*., 1995).

According to Manivannan *et al., (*2005), the determination of seminal fructose concentration is useful as a marker in the investigation of obstructive azoospermia (Absence of live sperm) and inflammation of male accessory glands. High seminal Fructose concentration is often seen in males with normal fertility (Buckett and Lewis-Jones, 2009).

Zinc (Zn) is a trace element that is essential for body functions. Its depletion could result in growth retardation, poor wound healing, acrodermatitis and infertility issues. Zn is seen in various food components. Despite this the WHO (1995) estimates that one-third of world population is deficient of Zn. Its content in semen is more than that in blood and studies have shown that Zn safeguards sperm from bacterial and chromosomal damage and plays an important role in normal testicular development, spermatogenesis and sperm motility (Khan *et al*., 2011).

Cadmium (Cd) is a soft, bluish-white metal that is found naturally in our environment. Cd has no known physiological function and so it is considered a toxicant. Many different forms of exposure to cadmium have been shown over the past century. Cd is present in the environment as a result of many human activities (Rahimzadeh *et al*., 2017).

The foundry industry generally has been regarded as a place prone to hazards; physical, mechanical and chemical hazards (e.g. toxic metals) and this can pose a serious threat to the health and safety of the workers. Most workers in non-formal vocations or small-scale enterprises usually have little or no occupational health knowledge and do not also take the use of PPE as necessary for protection against occupational hazards, so may be accumulating toxicants in the body that could result in impairment in their general body function (Igharo *et al*., 2018). Experimental animal and human occupational studies with increased exposure to toxic metals generally support a toxic effect of these metals in reproductive system (Meeker *et al*., 2008). Thus the aim of this study was to determine the level of fructose and zinc in seminal fluid and Cd/Zn in blood among Bronze foundry workers.

Materials and methods

Study design: The study was a cross-sectional study carried out in Benin City, South-south Nigeria. The groups composed of thirty-five (35) foundry workers (bronze casters) occupationally exposed and fifteen (15) age\sex-matched environmental participants (control).

Inclusion criteria: Workers who were occupationally exposed to bronze casting for a period of five years and above were included in the study while the environmental control subjects were healthy male individuals with no similar occupational exposure. Bronze casting is a male dominated vocation, so only males were recruited for the study.

Exclusion criteria: Participants with demographic or medical history of any form of disease, smoking and alcohol consumption were excluded from the study.

Ethical approval: The protocol for this study was approved by the Ethical Clearance Committee, Edo State Ministry of Health, with reference number HM.1208/7467.

Collection of data and sample collection: A validated questionnaire was administered to consenting participants to obtain social and demographic information.

Semen and blood collection: After abstinence of 3–5 days, semen samples from participants were collected in a sterile plastic container. Venous blood was collected using standard phlebotomy techniques.

Laboratory analysis:

Macroscopy and microscopy: Semen samples were examined after 30 min according to WHO (2010) criteria. Sperm cell indices such as sperm concentration, motility, and morphology were determined microscopically. Later, samples were centrifuged at 3000 rpm for 10 min and seminal plasma was stored at −20 °C for fructose estimation.

Biochemical assays:

Seminal fructose estimation: The estimation of fructose in semen was carried out using modified Karvonen method (1954). 20 µl of seminal plasma was mixed thoroughly with 220 µl distilled water, later it was

deprotinized with 50 µl of ZnSO4 and 50 µl of NaOH. After 15 min of incubation, it was centrifuged at 2500 rpm and 200 µl of clear supernatant was mixed with Indole reagent followed by 32% hydrochloric acid. The mixture was incubated at 60°C for 20 min and after cooling readings was taken at 470 nm.

Determination of metal levels in blood and seminal plasma Serum Zn, Cd and seminal Zn were determined using the inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Elemental, X series I, Germany), based on standard methods described by Fong *et al.* (2007).

Statistical analysis: Data collected were analyzed, interpreted and expressed in mean and standard error of the mean. Independent t-test was used to find whether the significant mean difference exists between the two groups. A p-value of less than 0.05 ($p < 0.05$) was considered significant.

Results

The anthropometric indices of the bronze casters and Environmental controls are shown in Table 1. From the observation the mean age of the bronze casters (46.67±1.23), and Environmental control (42.58±1.73) were not significantly different. ($p= 0.312$; $p> 0.05$). There were also no significant differences between height, weight and body mass index of the bronze casters and Environmental controls.

Table 1: Anthropometric indices of study Participants

Observation	Bronze Casters (M±SEM)	Environmental Control (M±SEM)	P-value
Age (years)	46.67±1.23	42.58±1.73	0.312
Sex	Male 100%	Male	
Height	1.71±0.01	1.71±0.01	0.987
Weight	74.7578±1.22674	71.92±1.61	0.308
BMI	25.67±0.42	24.87±0.69	0.525

Sperm cell indices between the bronze casters and environmental control group is shown in Table 2. From the observation, there was no significant difference in the two groups. The mean semen volume, sperm cell motility, Active sperm cell, Sluggish sperm cell, sperm cell morphology, Abnormality detected and Sperm concentration in the two study groups was not significantly different ($p> 0.05$).

Table 2: Sperm cell indices of study participants

Parameters	Test (M ± SEM) N=35	Environmental Control (M ±SEM) N=15	P-value
Volume	1.88 ±0.22	2.9 ±0.434	0.030
Motility (%)	30.6 ± 0.41	33.4 ±0.31	1.0
Active (%)	23 ±4.7	18 ± 4.6	0.457
Sluggish (%)	6.75 ± 1.12	12.73 ±2.62	0.49
Sperm Morphology (%)	27.45 ± 1.78	30.00 ± 1.69	0.226
Abnormality	27.45 ± 1.914	30.00 ± 1.690	0.242
Sperm Concentration	30.7±6.35	33.81 ± 2.27	0.792

$P< 0.05$= minimum significant

The mean values of selected seminal parameters (zinc and fructose) and plasma parameters (cadmium and zinc) is illustrated in Table 3. The blood cadmium was significantly higher in the Bronze casters than the environmental control group ($p<0.05$).There was significant difference between the bronze casters and environmental control group ($p<0.05$) for plasma concentration of Zinc. However there were no significant difference for seminal concentration of zinc and fructose ($p> 0.05$).

Table 3: Seminal and serum biomarkers of study participants

Parameters	Test (M ± SEM) N=35	Environmental Control (M ±SEM) N=15	P-value
Seminal Zinc (mmol/L)	1.22± 0.08	1.41±0.11	0.16
Fructose (mmol/L)	27.42±1.76	27.2 ± 1.87	0.935
Serum Cd (µg/ L)	0.99 ± 0.04	0.63 ± 0.02	0.00
Serum Zn (µg/ L)	86.02 ±2.56	104.52 ±3.11	0.00
Corrected fructose (mg/mL)	2.33 ±0.33	2.6 ± 0.25	0.567

Discussion

This study sought to determine the levels of blood Cd, Zn and seminal zinc and fructose, in bonze foundry workers and environmental participants (control). A major characteristic of a metal foundry is the melting and pouring of molten metals. This molten metal used in the foundry can be an alloy of metals. The commonly employed alloys have significant amounts of As, Cd, Cu etc (Dos Santos *et al.* 2015). Workers in such vocation can become occupationally exposed to toxic metals over time (Mgonja 2017). The result of this study showed a significant increase in the blood Cd of foundry workers when compared with the environmental control. This result is similar to what Freire *et al*. (2021) found in foundry workers. This result was also consistent with report obtained in Abuja involving automobile mechanics, generator and battery chargers (Alli, 2015). Cd is recognized as a endocrine disruptor by binding to androgen and estrogen receptors thereby inhibiting steroidogenesis and spermatogenesis, which may affect semen quality (Yeung, *et al*., 2011).

The age and sex frequency, and the BMI index were considerably similar in both groups, ruling out their effects on the outcome of data obtained.

Semen contains the major secretions from the reproductive organs; the seminal fluid contains 90% of secretions from the accessory organs and 10% of Sperm cells, which main purpose is to fertilize ova in the ampulla of the fallopian tube. Semen quality is used as a major forecaster and measure of male fertility outcome (Kumar and Singh, 2022). Sperm count and concentration have been strongly associated with the likelihood of pregnancy and has been known to be the cause of majority of male fertility problems (Leaver, 2016). The sperm cell indices of participants were not significantly different in both the foundry workers and the environmental control.

The motility of the sperm cell was of concerned because sperm cell motility of both the Bronze casters and Environmental control was poor as against the reference value by WHO set at 40% and this condition is referred to as Asthenozoospermia, the possible suggestive cause was the exposure of both groups to environmental toxicants in the foundry environment.

Zinc is an element that plays a protective function against cadmium aggregation, which is made possible by interaction between the two elements at various stages of absorption, distribution, excretion, and biological activity. In this study blood Zn level was higher in the environmental control than bronze casters. The possible reason why Zn level is low in the bronze caster is the known interactions between Cd and bioelements (Zn) leading to competition for the same binding sites on transport proteins that provide their entry into cells (Matović *et al*., 2010). Due to this competition Cd can displace Zn from most biological system through its competition with Zn for its transporter and also its displacement from p53 protein. This is similar to the findings by Lashari *et al*. (2021) in Pakistan where Cd and Zn interactions were seen in type1 diabetic children.

Rajalakhshmi *et al*. (1989) reported that fructose concentration in seminal plasma is one of the important markers of seminal vesicular function and if the seminal vesicular function is decreased, then semen coagulation, sperm motility, stability of sperm chromatin, and semen immune protection could be affected. However, Patel *et al*. (1988) demonstrated a positive correlation between seminal fructose concentration and percentage of sperm motility. Seminal fructose determined in this study was found to be within the reference value in both groups and there was no significant difference between them. The most common energy source for all spermatic activity is fructose, which is found in semen and produced by the Seminal vesicle. As a result, lower fructose concentrations may be seen in greater sperm concentrations (Lu *et al*., 2007).

When compared to environmental control, the observed amount of seminal zinc for bronze caster was lower. The mechanism behind the action of testosterone is stimulated by a higher zinc content, which also increases the number of germ cells in the lumen of seminiferous tubules and the effectiveness of the spermatogenic apparatus, as a result of binding of testosterone along with FSH to Sertoli cells receptors (Abdella *et al*., 2011).

Conclusion

This study has observed that foundry workers had increased blood Cd than those of the environmental control group. However, seminal fructose and zinc levels in our research sample were within acceptable limits for adults male. As a consequence, with continued exposure, cadmium buildup in the body causes poor sperm cell density and volume as well as a rise in the number of immature sperm cells.

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