International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Sharma N et al. Int J Reprod Contracept Obstet Gynecol. 2025 Jul;14(7):2200-2208
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20251966

Original Research Article

Metallothionein gene polymorphism associated with Cd and Hg levels in non-obstructive azoospermia patients: a hospital-based observational study

Nisha Sharma[1], Ashutosh Halder[1], Seema Kaushal[2], Manoj Kumar[3], Manish Jain[1]*

[1]Department of Reproductive Biology, All India Institute of Medical Sciences, Delhi, India
[2]Department of Pathology, All India Institute of Medical Sciences, Delhi, India
[3]Department of Urology, All India Institute of Medical Sciences, Delhi, India

Received: 09 April 2025
Revised: 26 May 2025
Accepted: 27 May 2025

***Correspondence:**
Dr. Manish Jain,
E-mail: manishjain@aiims.edu

ABSTRACT

Background: Heavy metals, such as Cadmium (Cd), Arsenic (As), Mercury (Hg) and Lead (Pb) might potentially induce reproductive toxicity in male infertility patients, regardless of the varying concentrations of these heavy metals in the blood. Genetic polymorphism is one of the least studied internal contributing factors in male infertility cases associated with high level of heavy metal in blood. Therefore, this study aims at identifying the difference in the serum levels of heavy metals in non-obstructive azoospermia (NOA) patients associated with genetic variants.
Methods: It's a hospital based observational study where patients reporting with azoospermia due to hypospermatogenesis (HS) were recruited prospectively. Comprehensive clinical history, and blood samples were collected. Whole exome sequencing (WES) and was performed for 50 HS patients to identify variants. Inductively coupled plasma mass spectrometry (ICP-MS) was performed to assess levels of Cd, As, Hg and Pb levels in serum samples of 50 HS patients. Statistical analysis was performed to determine difference in heavy metal concentration of HS patients with and without the presence of metallothionein gene associated single nucleotide polymorphism (SNP).
Results: Genomic analysis for SNPs identified deleterious candidate variants in MT1A (rs11640851 and rs8052394) associated with 18/50, MT1E (rs138690474) associated with 4/50 and MT4 (rs11643815) associated with 5/50 HS patients. A statistically significant difference in the blood concentration of Cd and Hg was observed in HS patients associated with metallothionein gene SNPs.
Conclusions: This exploratory genomic analysis conducted on HS patients shows prevalence of deleterious candidate SNPs in metallothionein gene. The HS patients with candidate SNPs showed higher levels of Cd and Hg which indicate the genomic susceptibly towards heavy metal-induced reproductive toxicity.

Keywords: Azoospermia, Hypospermatogenesis, Copy number variants, Heavy metals, Cadmium, Mercury, Male infertility

INTRODUCTION

It is estimated that 48.5 million couples, which constitute 15% of all couples across the world are infertile.[1] Previous studies have reported that in 20-30% of infertility cases, male partners play a major role. The male reproductive system is susceptible to various occupational and environmental factors, only a handful of which are thoroughly studied. Amongst all, heavy metals have been identified as a major factor for male infertility.[2] These heavy metals are considered "endocrine disrupting chemicals" (EDCs) as they interfere with the body's

endogenous hormone-regulated systems, hence impairing endocrine functions.[3] The primary source of heavy metals is considered to be contaminated food.[4] Exposure to heavy metals causes a build-up in the body since the human body lacks the metabolic processes necessary to remove them. Over the past century, there has been a growing interest in the health and developmental hazards of heavy metal exposure. Because heavy metals reduce the quality of the seminal fluid, they can impact male fertility and result in infertility.[5]

Genetic polymorphism is one of the least studied internal contributing factors in male infertility cases associated with high levels of heavy metals in the blood. Prior research has documented a connection between specific gene polymorphisms of metallothioneins (MTs) and the levels of cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), copper (Cu), zinc (Zn), selenium (Se) and iron (Fe), in the bodies of healthy and diseased individuals.[6] Metallothioneins are a superfamily of intracellular, metal-binding proteins that are rich in cysteines and found in eukaryotes, prokaryotes, plants, vertebrates, and invertebrates.[6] MTs are characterized by their low molecular mass (6–7 kDa), high cysteine content (about 30%), lack of aromatic amino acids, and strong metal binding affinity, especially for Zn, Cu, and Cd. Depending on the tissue type, the thiol (-SH, mercaptides) group's metal binding ratios and quantities vary. There are four primary categories of MTs in mammals, including humans: MT1, MT2, MT3, and MT4. These groups differ in their expression, sequence, and characteristics.[7]

Even at low levels, the hazardous, bioaccumulative, non-essential, and widely distributed heavy metal cadmium has several identified adverse health effects.[8] Humans are most exposed to Cd through their gastrointestinal or respiratory tracts; food, drinking water, air, and cigarette smoke are significant non-industrial exposure sources. Because of its propensity to accumulate, Cd has a half-life of 10 to 30 years in the human body. As a result, tissue concentrations of this metal rise throughout a lifetime with ongoing environmental exposure. Following absorption, the Cd is quickly carried to the liver by the blood, where it binds to MT. The glomerulus freely filters the Cd bound to MT, and the renal tubule effectively reabsorbs this complex.[9] Mercury (Hg) is a hazardous heavy metal that has detrimental impacts on both the environment and human health.[10-12] Because of its pervasiveness and adverse effects, it is currently ranked among the top three substances of concern for public health.[13] A variety of human activities release mercury into the atmosphere, with artisanal and small-scale gold mining (ASGM) being the primary source of emissions.[12,14] Humans are commonly exposed to mercury over an extended period by consuming contaminated food, particularly fish, which is a major source of protein for many vulnerable groups around the world.[15,16] Male fertility is adversely affected by mercury.[17] It has been previously reported that Mercury causes DNA breakage in spermatozoa, which reduces sperm motility, dysfunction, and viability.[17,18] Numerous animal studies have documented the reproductive toxicity of mercury, showing that exposure to the metal reduced sperm motility, epididymal sperm count, and normal sperm morphology in rats, mice, and monkeys.[19,20] It has been noted that abnormal sperm motility and morphology are associated with seminal fluid mercury concentrations; infertile and sub-fertile men have been found to have higher mercury levels than fertile men; and infertile patients exposed to mercury have been found to exhibit tubular atrophy and Sertoli-cell-only syndrome.[21-24] The SNPs in genes involved in Hg toxicity are predicted to be linked with adverse Hg and Cd poisoning and may help identify susceptible subgroups at risk in exposed populations.

METHODS

Patient recruitment and laboratory procedures

This was a hospital based observational study, conducted at the Department of Reproductive Biology, All India Institute of Medical Sciences-New Delhi, India for three years from March 2020 to March 2023. Male infertile patients who visited the urology department were enlisted, and each of them underwent a physical examination, and clinical history was taken. Fertile controls were recruited from the general population and blood samples were collected. Informed, written consent was obtained from the participants and the study was performed according to the Declaration of Helsinki. The study was conducted in compliance with the regulations and approval from the All India Institute of Medical Sciences ethical committee in Delhi, India; with the approval ID, IEC-151/06.03.2020, RP-17/2020. The hormonal evaluation of infertile men was performed using chemiluminescent based assay. The levels of Serum follicle-stimulating hormone (FSH), serum luteinizing hormone (LH), and serum testosterone were measured using kits from Abbott Laboratories' second generation. The semen analysis of all participants was performed following WHO criteria 2021. The ejaculate was collected in a pre-weighed container and the volume of the semen was measured. The semen sample was liquefied on incubation for 30 minutes and the pH was measured. To determine the fructose content, the semen sample was heated in strong acid and resorcinol. Further, the sample was evaluated under a microscope for spermatozoa at 10X and 40X magnifications. For spermatozoa >= 101 per field at 40× magnification, a 1:20 dilution ratio was chosen to count sperm in 50 µl of well-mixed ejaculate in 950µL of fixative. Neubauer's chamber was used for microscopic counting. Cases with no observed sperm post centrifugation and a sperm count <2.5 mio/ml were considered azoospermia and oligozoospermia respectively. The patients with obstructive causes were excluded and a bilateral testicular fine needle aspiration evaluation was performed for confirmatory diagnosis of non-obstructive azoospermia (NOA).

Identification of HS cases

Azoospermic and oligozoospermic men with normal or slightly altered reproductive endocrine parameters were advised for testicular FNA evaluation to be categorized as hypospermatogenesis (HS). The testicular Fine needle aspiration (FNA) procedure followed by reporting was done at the Department of Pathology. For sample collection, a spermatic block containing 1% lignocaine was given to the patients. A 22-gauge needle attached to a 10 ml syringe on a plunger was used at two distinct locations to get the aspirates from the right and left testicles. A thread-like structure in the aspirate was used to prepare smears before staining. Every smear was confirmed for the presence of at least 2000 cells. FNA from both the testes showing a considerable decrease in the spermatogenic series of cells per Sertoli cell, observed till maturation to spermatozoa, is suggestive of HS.

Identification of idiopathic HS cases

A comprehensive clinical history of the HS patients was taken to investigate male factor infertility. Patients were excluded if they had conditions such as the presence of hydrocele or varicocele, history of testicular trauma, testicular maldescent, and history of radiotherapy, chemotherapy, or exposure to any reproductive toxin and orchitis. The blood samples of the 59 participants (Supplementary sheet 1) were collected in EDTA vials for DNA isolation using the QIAamp DNA Blood Mini Kit (QIAGEN, 51106) according to the manufacturer's instructions. The optical density of the isolated DNA was measured using a spectrophotometer (Nanodrop). Karyotyping was done for the metaphase stage in heparinized blood samples of all 59 HS patients, using karyotyping medium (Thermofisher Ref 12557-013). In-house X and Y centromere probes were used for XY FISH analysis on metaphase cells. Signals from X- and Y-centromeric probes were enumerated in a minimum of five metaphase cells. Multiplex sequence tagged sites (STS) PCR analysis for Yq microdeletion was carried out for 46 HS cases using AZFa- sY84, sY86; AZFb -sY127, sY134; AZFc -sY254, sY255, and SRY primers. The PCR amplicon was visualized by running on a 4% agarose gel. A total of nine HS cases were were excluded due to presence of known genetic cause associated with them, amongst which microdeletions of the Y chromosome were found in four patients, and aneuploidies of the sex chromosomes by FISH analysis were observed in five patients. Finally, a total of 50 HS cases were selected for the WES and ICP-MS study.

WES and data analysis

Sequencing libraries were prepared using SureSelectXT Human All Exon (SSV5+UTRs). The enriched DNA libraries were multiplexed by adding index tags by amplification, followed by purification. Prepared libraries were sequenced on an Illumina HiSeqX to generate 100X target coverage, and 2x150 bp reads/sample. Approximately 75% of the sequenced bases had Q30 values. The sequence was processed to generate FASTQ files. Base trimming was performed using a custom script, and open-source software, such as cutadapt or fastq-mcf, was used for adapter trimming. Read alignment was performed using the hg19 version of the BWA aligner. PCR-duplicate reads were removed using the Picard toolkit. Reads were realigned around the known indels using the Genome Analysis Toolkit (GATK – Indel Realigner). Both haplotype caller and genotype caller variant files were merged into a single variant VCF file. A detailed tertiary analysis and filtering of whole exome sequencing data to isolate single nucleotide polymorphism is given in one of our previous publication.[25]

ICP-MS: Inductively coupled plasma mass spectrometry

Sample preparation

The EDTA sample tubes were kept at 4 °C for 2 hours before the digestion of the whole blood sample. The whole blood samples were digested in a microwave digester (Multiwave 5000, Anton Paar). Approximately, 0.5 g of whole blood sample was was weighed in PTFE tubes followed by the addition of 3 ml of nitric acid (69 % HNO_3 suprapur grade) through the wall of the digester. Digester tubes were left open for 1-2 minutes and then 1ml of hydrogen peroxide (30 % H_2O_2 suprapur grade) was added in the sample for complete digestion. The digestion vessels were left open for 10 minutes to complete the reaction. The vessels were closed tightly and placed into the digester. The temperature of the digester was set at 170°C for 20 minutes and was on hold for 20 minutes. After the digestion was completed, the vessels were left for 30 minutes to cool down. Samples were transferred into sample tubes and the volume was made up to 50 ml.

Sample analysis

Heavy metal analysis in whole blood samples is done by inductively coupled plasma mass spectrometer (ICP-MS) (NEXION 1000, Perkin Elmer). The instrument is calibrated at five concentration points using multi-element standards from Merk. The calibration curve (linear through zero) plotted between signal intensity and standard concentration showed a regression coefficient R2 of 0.999. After calibration, internal calibration verification standard is checked which showed a recovery of 95-103%. After achieving a significant recovery, all the samples were analyzed. After the run of 10 samples, one quality control (QC) sample is analyzed to know and minimize the carryover and matrix effect.

Statistical analysis

The statistical analysis was done using Graphpad prism. Descriptive analysis was performed to determine mean and standard error followed by unpared T-test with Welch's correlation to assess the difference in the levels of heavy metal among cases and controls. Fold change

calculation was performed in order to compare the Cd and Hg levels among cases and fertile control groups.

RESULTS

Metallothionein SNPs found in HS patients

On genomic analysis, we found missense mutation in the three genes (MT1A NM_005946.3, MT1E NM_001363555.2 and MT4 NM_032935.3). The mutations identified were Thr27Asn and Lys51Arg in the gene MT1ANM_005946.3; Lys51Arg in the gene MT1ENM_001363555.2 and Cys21Ser in the gene MT4NM_032935.3. SNP, rs11640851 was identified in the gene, MT1ANM_005946.3 with Thr27Asn mutation and SNP, rs8052394 in the same gene having Lys51Arg mutation. The SNP, rs11640851 showed heterozygosity in 14/50 patients compared to homozygosity in 4/50 patients and was predicted to be 'deleterious'. The SNP, rs8052394 identified in all the 5/50 patients were in heterozygous form with the prediction of being 'deleterious' by CADD_phred and 'damaging' by SIFT. The SNPs rs138690474 and rs11643815 were identified as associated with the genes MT1E NM_001363555.2 and MT4 NM_032935.3 respectively. Both were found to be in the heterozygous form in 4/50 and 5/50 patients respectively. The SNP rs138690474 was predicted to be 'deleterious' by CADD_phred, 'probably damaging' by PolyPhen and 'tolerated' by SIFT. On the other hand, rs11643815 was dominantly predicted to be 'deleterious' (Table 1).

Table 1: Characteristics of deleterious SNPs associated with metallothionein genes found in HS patients.

Gene	Mutation type	HGVS	rs_dbSNP	Zygosity	SIFT	PolyPhen	CADD_phred	Frequency
MT1A NM_005946.3	Missense	c.80C>A p.Thr27Asn	rs11640851	Het Hom	0.008 (Deleterious)	0.928 (Possibly damaging)	23 (Deleterious)	Nhet = 14/50 Nhom= 4/50
MT1A NM_005946.3	Missense	c.152A>G p.Lys51Arg	rs8052394	Het	0.80649 (Damaging)	NA	26 (Deleterious)	Nhet= 5/50
MT1E NM_001363555.2	Missense	c.61T>A p.Cys21Ser	rs138690474	Het	0.027 (Tolerated)	0.975 (Probably damaging)	26 (Deleterious)	Nhet= 4/50
MT4 NM_032935.3	Missense	c.143G>A p.Gly48Asp	rs11643815	Het	0.127 (Deleterious)	1 (Probably damaging)	26 (Deleterious)	Nhet= 5/50

MT1A= metallothionein 1 A; MT1E= metallothionein 1 E; MT4= metallothionein 4; HGVS c.=human genome variation society coding DNA reference sequence; HGVS p.= human genome variation society protein change; rs ID=reference single nucleotide polymorphism id; Het=heterozygous; Hom=Homozygous; SIFT= Sorting Intolerant from Tolerant; PolyPhen= Polymorphism Phenotyping; CADD= combined annotation-dependent depletion

Table 2: The Allele frequency of identified SNPs in various population database.

Variant Id	Allele frequency database						
	1000Genomes	**Allele frequency aggregator**	**ExAC**	**gnomAD**	**HapMap**	**Genome-wide autozygosity in Daghestan**	**AF in gnomAD (Asian) vs HS cohort**
MT1A rs11640851	Global=0.0563 South Asian=0.072	Global=0.115968 South Asian=0.065	Global=0.1108 Asian=0.06568	Global=0.10756 Asian=0.51072	Global=0.0599 Asian=0.00	Global=0.1336 South Asian=0.09	(25022/48994) Vs (18/50) 0.036
MT1A rs8052394	Global=0.1765 South Asian=0.184	Global=0.13495 South Asian=0.18	Global=0.14667 Asian=0.18627	Global=0.14762 Asian=0.18869	NA	NA	(9246/49002) Vs (5/50) 0.1
MT1E rs138690474	Global=0.0034 South Asian=0.017	Global=0.00065 South Asian=0.01	Global=0.00334 South Asian=0.014	Global=0.00295 South Asian=0.01361	NA	NA	(667/49010) Vs 4/50 0.08

Continued.

Variant Id	Allele frequency database						
	1000Genomes	Allele frequency aggregator	ExAC	gnomAD	HapMap	Genome-wide autozygosity in Daghestan	AF in gnomAD (Asian) vs HS cohort
MT4 rs11643815	Global=0.0563 South Asian=0.072	Global=0.115968 South Asian=0.065	Global=0.11080 South Asian=0.06568	Global=0.10756 South Asian=0.0610	Global=0.0599 South Asian=0.000	Global=0.1336 South Asian=0.09	2918/47848 Vs 5/500.1

Table 3: The difference in blood levels of Cd, As, Hg and Pb between cases and controls.

Variables	Cases (n=23)		Control (n=27)		P value
	Mean	SE Mean	Mean	SE Mean	
Cd µg/kg	0.406	0.28	-0.25	0.26	<0.01**
As µg/kg	3.49	0.25	3.37	0.145	0.053
Hg µg/kg	1.02	0.31	0.54	0.21	<0.01**
Pb µg/kg	0.12	0.0084	0.12	0.010	>0.9999

Cd µg/kg=normalized concentration of Cadmium in whole blood samples; As µg/kg= normalized concentration of Arsenic in whole blood samples; Hg µg/kg= normalized concentration of Mercury in whole blood samples; Pb µg/kg: normalized concentration of Lead in whole blood samples; Mean= geometric mean of normalized concentrations of all four heavy metals; SE Mean=Standard error of mean;(**)=calculation of difference using student T-test with P-value<0.01.

Table 4: The clinical characteristics of HS patients with (cases) and without (controls) SNPs.

Variables	Cases (n=23) (Mean ± SE)	Controls (n=27) (Mean ± SE)	P value
Age	30.88±4.46	29.66±3.43	0.2910
Duration of infertility	5.63±2.99	5.06±2.54	0.4757
FSH mIU/ml	12.72±2.07	10.26±1.47	<0.05*
LH mIU/ml	5.97±0.46	5.08±0.44	<0.05*
Serum Testo. ng/ml	4.40±0.29	5.90±0.98	<0.05*
BMI Kg/m^2	25.032±4.23	23.98±2.17	0.2901

Serum FSH= serum follicle stimulating hormone (reference range=0.95-11.95mIU/ml); Serum LH= serum luteinizing hormone (reference range=0.57-12.07mIU/ml); Serum T= serum testosterone (reference range=1.42-9.23 ng/ml); BMI=Body mass index. *Statistically significant.

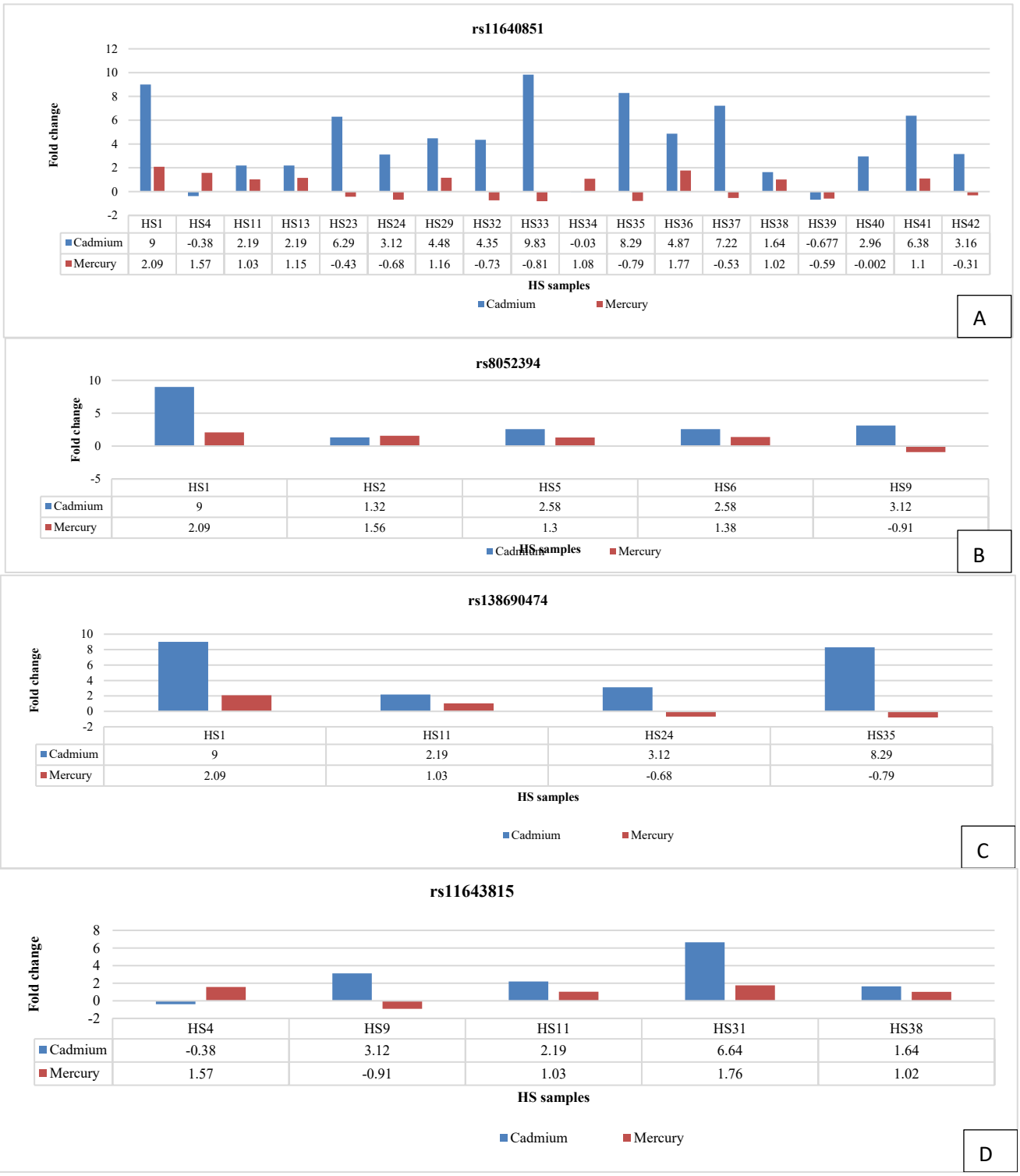
Allele frequency in population database

The allele frequency (AF) of the identified SNPs was collected using different databases. The databases used were 1000Genomes, Allele frequency aggregator, ExAC, gnomAD and HapMap. Rare alleles are defined as single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) of less than 0.01. They are considered polymorphic alleles with less than 1% frequency. We compared the AF of the four different SNPs in the global and South Asian population using five databases. As per the definition discussed above, the MAF was observed in the SNP, rs138690474 under the gene, MT1E in both the Global and South Asian population. When compared with the Asian populations, MAF was not observed in any of the SNPs in the HS cohort. The detailed analysis is described in Table 2.

Assessment of levels of heavy metals in serum

We measured the levels of the four heavy metals, Cd, As, Hg and Pb in the blood serum of the HS patients. Next, the levels in the 23 patients with SNPs were compared with the 27 patients identified without SNPs. We observed a significant increase in the serum levels of Cd and Hg in the patients identified with SNPs compared to patients without SNPs. In contrast, there was no significant difference in the levels of As and Pb among the groups. The levels of the heavy metals and the statistical analysis are elaborated in Tables 3. The Cd and Hg levels of cases with SNPs and fertile control group were assessed in terms of fold change (Figure 1a-d). We observed a maximum fold change of 9.83 and 2.09 and a minimum fold change of -0.38 and -0.81 in Cd and Hg levels respectively in the patients identified with SNP, rs11640851 (Figure 1a). Next, in the cases with SNPs, rs8052394 and rs138690474, the maximum fold change of Cd levels was 8.29 and Hg levels was 2.09 respectively (Figure 1b and 1c). We observed that a single patient, HS1 was detected with all the three above mentioned SNPs and showed the maximum fold change in Hg levels amongst the cases with the three SNPs individually. Further the fold change in his Cd levels was the highest in the groups of patients detected with SNPs, rs8052394 and rs138690474 individually compared to the

fertile control group. The minimum fold change of Cd and Hg levels in the group with SNP, rs8052394 were 1.32 and -0.91 respectively and in the cases with the SNP, rs138690474 were 2.19 and -0.68 respectively (Figure 1b and 1c). Lastly in the cases with SNP, rs11643815, a maximum fold change of 6.64 and 1.76 was observed in the levels of Cd and Hg respectively. The minimum fold change in the Cd and Hg levels in the same group were -0.38 and -0.91 respectively (Figure 1d).



rs11640851	HS1	HS4	HS11	HS13	HS23	HS24	HS29	HS32	HS33	HS34	HS35	HS36	HS37	HS38	HS39	HS40	HS41	HS42
Cadmium	9	-0.38	2.19	2.19	6.29	3.12	4.48	4.35	9.83	-0.03	8.29	4.87	7.22	1.64	-0.677	2.96	6.38	3.16
Mercury	2.09	1.57	1.03	1.15	-0.43	-0.68	1.16	-0.73	-0.81	1.08	-0.79	1.77	-0.53	1.02	-0.59	-0.002	1.1	-0.31

rs8052394	HS1	HS2	HS5	HS6	HS9
Cadmium	9	1.32	2.58	2.58	3.12
Mercury	2.09	1.56	1.3	1.38	-0.91

rs138690474	HS1	HS11	HS24	HS35
Cadmium	9	2.19	3.12	8.29
Mercury	2.09	1.03	-0.68	-0.79

rs11643815	HS4	HS9	HS11	HS31	HS38
Cadmium	-0.38	3.12	2.19	6.64	1.64
Mercury	1.57	-0.91	1.03	1.76	1.02

Figure 1 (A-D): The fold change in the levels of mercury and cadmium in the HS patients identified with the SNPs, rs11640851, SNPs, rs8052394, rs138690474 SNP, and rs11643815 is described. The fold change was calculated in comparison to the fertile control group.

Demographic characteristics of HS patients with and without SNPs

On comparison of the clinical characteristics of HS patients with and without SNPs, we observed a significant increase in Follicle Stimulating Hormone (FSH) and Luteinizing hormone (LH) in the patients identified with SNPs. Further, a significant decrease in serum testosterone was detected in the same group of patients. The clinical characteristics of the participants are described in Table 4.

DISCUSSION

Male infertility, a common problem nowadays, is impacted by lifestyle, environmental, and genetic factors. Reproductive health has been linked to exposure to heavy metals like Cd, Pb, As and Hg, among other environmental concerns. Metallothioneins (MTs) are cysteine-rich proteins that cause detoxification and regulation of heavy metals in the body. It is reported that polymorphism in the MT genes can impact the expression and function of the protein, which may increase vulnerability to metal toxicity and associated reproductive problems.[26] Inorganic arsenic, cadmium, lead, and mercury have been shown in numerous studies to have adverse impacts on the epididymis, which is essential for sperm maturation. According to reports, exposure to heavy metals decreased sperm motility, sperm quantity, and epididymal weight. Lead, cadmium, and inorganic arsenic harmed sperm structures in the epididymal duct.[27] In a recent case-control study published by Hassan et al, it has been described that compared to controls, infertile males had significantly higher blood and semen Cd levels, lower-quality semen, more oxidative stress, and higher MT1A methylation.[28] In our study, we also observed a similar pattern. The HS patients identified SNPs in MT1 and MT4 genes had significantly higher blood levels of Hg and Cd. Moreover, this group of patients also showed significantly higher levels of FSH and LH with significantly lower serum testosterone levels. Perini et al reported that the SNP, Rs8052394 was associated with both higher levels of Hg and neurotoxicity.[29] This SNP is also reported to be associated with type 2 diabetes mellitus.[30] On a similar note, we observed this SNP to be associated with male infertility and higher blood levels of Cd and Hg. In addition to this Yang et al in their study have shown the association of MT1A SNPs (rs11640851 and rs8052394) with blood lead levels and nephrotoxicity.[31] In contrast, no significant change in blood lead levels was observed in the HS patients identified with these SNPs in our study. The significant rise in Cd and Hg levels in SNP-affected patients is consistent with earlier studies showing that genetic variables might affect heavy metal metabolism and excretion. Increased levels of these metals seem to be associated with the SNPs under investigation, specifically rs11640851, rs8052394, and rs138690474. This could be a sign of compromised detoxification pathways or changed absorption processes. The observed rises in serum concentrations could be caused by these genetic differences altering the expression or function of proteins involved in metal transport and detoxification. It's interesting to note that our investigation revealed no significant differences in the blood levels of lead (Pb) and arsenic (As) in the HS cases compared to the fertile control. This may indicate that As and Pb are not affected by the same genetic factors that affect the metabolism of Cd and Hg, underscoring the complexity of heavy metal toxicity and the need for more research into the mechanisms underlying their accumulation.

Limitation

Considering limited reports on the association of Metallothionein I gene polymorphism with the accumulation of heavy metals in infertile males, our study can be considered as preliminary research in this direction. The disadvantage of this study was the small sample size. However, this study demands the necessity of more research to explore the chances of MT1A SNPs (rs11640851 and rs8052394), MT1E SNP (rs138690474) and MT4 (rs11643815) as biomarkers for infertile males with high blood levels of Cd and Hg.

CONCLUSION

Thus, it can be concluded that the HS cases detected with SNPs rs11640851, rs8052394, rs138690474 and rs11643815 showed significant increased blood levels of Cd and Hg. Thus, these SNPs can be considered as potential biomarker candidates for the HS patients with higher blood levels of heavy metals.

ACKNOWLEDGEMENTS

A sincere thanks to Indian Council of Medical Research for providing funding for this work. The technical staff of Department of Reproductive Biology, molecular cytogenetics laboratory provided support for Yq microdeletion and XY-FISH.

Funding: The work is funded by Indian Council of Medical Research (ICMR), India
Conflict of interest: None declared
Ethical approval: Ethical approval was obtained from the institute ethical committee of All India Institute of Medical Sciences in Delhi, India (IEC-151/06.03.2020, RP-17/2020)

REFERENCES

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod. Biol. Endocrinol. 2015;13:1-9.
2. Figa-Talamanca I. Traina ME, Urbani E. Occupational exposures to metals, solvents and pesticides: Recent evidence on male reproductive effects and biological markers. Occup Med. 2001;51:174-88.
3. Kopp R, Martínez IO, Legradi J, Legler J. Exposure to endocrine disrupting chemicals perturbs lipid

metabolism and circadian rhythms. J Environ Sci. 2017;62:133-7.
4. Balabanic D, Klemencic AK. Diet containing endocrine-disruptors and reproductive health. Handbook of Diet and Nutrition in the Menstrual Cycle. J Reprod Fertil. 2014;2014:359–72.
5. Balabaniˇc D, Rupnik MS, Klemenˇciˇc AK. Negative impact of endocrine-disrupting compounds on human reproductive health. Reprod Fertil Dev. 2011;23:403–16.
6. Sekovanić A, Jurasović J, Piasek M. gene polymorphisms in relation to diseases and trace element levels in humans. Archives of Industrial Hygiene and Toxicology. 2020;71(1):27-47.
7. Kimura T, Kambe T. The functions of metallothionein and ZIP and ZnT transporters: an overview and perspective. Int J Mol Sci. 2016;17:336.
8. Pan J, Plant JA, Voulvoulis N, Oates CJ, Ihlenfeld C. Cadmium levels in Europe: implications for human health. Environ. Geochem. Health. 2010;32:1–12.
9. Kayaaltı Z, Aliyev V, Söylemezoğlu T. The potential effect of metallothionein 2A− 5 A/G single nucleotide polymorphism on blood cadmium, lead, zinc and copper levels. Toxicol Applied Pharmacol. 2011;256(1):1-7.
10. Arrifano GP, Augusto-Oliveira M, Lopes-Araujo A, Santos-Sacramento L, Macchi BM, Nascimento J, et al. Global Human Threat: The Potential Synergism between Mercury Intoxication and COVID-19. Int J Environ Res. Public Health. 2023;20:4207.
11. Arrifano GP, Crespo-Lopez ME, Lopes-Araujo A, Santos-Sacramento L, Barthelemy JL, de Nazare CGL, et al. Neurotoxicity and the Global Worst Pollutants: Astroglial Involvement in Arsenic, Lead, and Mercury Intoxication. Neurochem Res. 2023;48:1047–65.
12. Crespo-Lopez ME, Barthelemy JL, Lopes-Araújo A, Santos-Sacramento L, Leal-Nazaré CG, Soares-Silva I, et al. Revisiting Genetic Influence on Mercury Exposure and Intoxication in Humans: A Scoping Review. Toxics. 2023;11(12):967.
13. Agency for Toxic Substances and Disease Registry. ATSDR 2022 Substance Priority List. Available at: https://www.atsdr.cdc.gov/spl/index.html. Accessed on 17 May 2023.
14. Crespo-Lopez ME, Augusto-Oliveira M, Lopes-Araújo A, Santos-Sacramento L, Souza-Monteiro JR, da Rocha FF, et al. Mercury Neurotoxicity in Gold Miners. In: Lucchini RG, Aschner M, Costa LG, editors. Advances in Neurotoxicology. Cambridge (MA): Academic Press; 2022;7:283–314.
15. Machado CLR, Crespo-Lopez ME, Augusto-Oliveira M, Arrifano GP, Macchi BM, Lopes-Araujo A, et al. Eating in the Amazon: Nutritional Status of the Riverine Populations and Possible Nudge Interventions. Foods. 2021;10:1015.
16. Rodriguez Martin-Doimeadios RC, Berzas Nevado JJ, Guzman Bernardo FJ, Jimenez Moreno M, Arrifano GP, Herculano AM, et al. Comparative Study of Mercury Speciation in Commercial Fishes of the Brazilian Amazon. Environ Sci Pollut Res Int. 2014;21:7466–79.
17. Mocevic E, Specht IO, Marott JL, Giwercman A, Jönsson BA, Toft G, et al. Environmental Mercury Exposure, Semen Quality and Reproductive Hormones in Greenlandic Inuit and European Men: A Cross-Sectional Study. Asian J Androl. 2013;15(1):97.
18. Arabi M, Heydarnejad MS. In vitro mercury exposure on spermatozoa from normospermic individuals. Pak J Biol Sci. 2007;10:2448–53.
19. Boujbiha MA, Hamden K, Guermazi F, Bouslama A, Omezzine A et al. Testicular toxicity in mercuric chloride treated rats: association with oxidative stress. Reprod Toxicol. 2009;28:81–9.
20. Homma-Takeda S, Kugenuma Y, Iwamuro T, Kumagai Y, Shimojo N. Impairment of spermatogenesis in rats by methylmercury: involvement of stage- and cell-specific germ cell apoptosis. Toxicol. 2001;169:25–35.
21. Choy CM, Lam CW, Cheung LT, Briton-Jones CM, Cheung LP et al. Infertility, blood mercury concentrations and dietary seafood consumption: a case–control study. BJOG. 2002;109:1121–5.
22. Basta PC, Viana PVDS, Vasconcellos ACSD, Périssé ARS, Hofer CB, Paiva NS, et al. Mercury Exposure in Munduruku Indigenous Communities from Brazilian Amazon: Methodological Background and an Overview of the Principal Results. Int J Environ Res Public Health. 2021;18:9222.
23. Silva MC, de Oliveira RAA, Vasconcellos ACS, Rebouças BH, Pinto BD, Lima MO, et al. Chronic Mercury Exposure and GSTP1 Polymorphism in Munduruku Indigenous from Brazilian Amazon. Toxics. 2023;11:138.
24. Andreoli V, Sprovieri F. Genetic aspects of susceptibility to mercury toxicity: An overview. Int J Environ Res Public Health. 2017;14:93.
25. Sharma N, Halder A, Kaushal S, Kumar M, Jain M. Identification of deleterious variants associated with male infertility genes in a cohort of idiopathic hypospermatogenesis patients. Frontiers In Reproductive Health. 2025;6:1494585.
26. Kamiński P, Baszyński J, Jerzak I, Kavanagh BP, Nowacka-Chiari E, Polanin M, et al. External and Genetic Conditions Determining Male Infertility. Int J Mol Sci. 2020;21(15):5274.
27. Machado-Neves M. Effect of heavy metals on epididymal morphology and function: An integrative review. Chemosphere. 2022;291:133020.
28. Hassan J, Elmetwalli A, Helal M, Al Munajer EA, Hussien TM, Saad AAA, et al. Cadmium Exposure and Its Association with Oxidative Stress, MT1A Methylation, and Idiopathic Male Infertility in Egypt: A Case-Control Study. Food Chem Toxicol. 2024;192:114925.
29. Perini JA, Cardoso JV, Knesse ADO, Pessoa-Silva FO, Vasconcellos ACSD, Machado DE, et al. Single-Nucleotide Polymorphisms Associated with Mercury

Levels and Neurological Symptoms: An Overview. Toxics. 2024;12(3):226.
30. Yang L, Li H, Yu T, Zhao H, Cherian MG, Cai L, et al. Polymorphisms in Metallothionein-1 and -2 Genes Associated with the Risk of Type 2 Diabetes Mellitus and Its Complications. Am J Physiol Endocrinol Metab. 2008;294(5):E987–92.
31. Yang CC, Lin CI, Lee SS, Wang CL, Dai CY, Chuang HY. The Association of Blood Lead Levels and Renal Effects May Be Modified by Genetic Combinations of Metallothionein 1A 2A Polymorphisms. Sci Rep. 2020;10(1):9603.

Cite this article as: Sharma N, Halder A, Kaushal S, Kumar M, Jain M. Metallothionein gene polymorphism associated with Cd and Hg levels in non-obstructive azoospermia patients: a hospital-based observational study. Int J Reprod Contracept Obstet Gynecol 2025;14:2200-8.