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Original Article

Orthopaedics Section

High Serum Estradiol and Heavy Metals Responsible for Human Spermiation Defect-A Pilot Study

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ABSTRACT

Introduction: Spermiation is a process of releasing sperm into the lumen of seminiferous tubules. Failure in releasing sperm into the lumen is designated as spermiation defect. Spermiation defect cases present as oligo-azoospermia or azoospermia despite normal gonadotropins and testicular histology/cytology. Human spermiation defect was never got attention to investigate in infertility practice. Most of the information on spermiation defect so far is from animal experiments. We assume some cases of non-obstructive azoospermia with normal gonadotropins and testicular histology/cytology could be due to spermiation defect.

Aim: The aim of the study was to find out the underlying aetiology in cases of human spermiation defect.

Materials and Methods: A total of 13 cases of spermiation defect and 20 fertile men as control constituted study material. Cases were studied for chromosomal abnormalities by conventional karyotyping, sex chromosome mosaicism by interphase XY FISH, Yq microdeletion by STS PCR, sertoli cell quality (function) and quantity (numbers) by serum Anti-Mullerian Hormone (AMH) and inhibin B besides other hormones like Follicular Stimulating Hormone (FSH), prolactin, testosterone and estradiol. Vitamin A concentration in serum was also measured. Presence of heavy metal was investigated by elemental electron microscopy in seminal cells (eight cases) & by spectrometry in serum as well as seminal plasma.

Results: Chromosomal and Yq microdeletion study failed to detect any abnormalities. AMH, inhibin B and vitamin A were also normal. Estradiol level was high in 6 out of 13 cases (46%) while platinum in seminal cells was high in 4 cases (50%). High (four times or more) serum level of lead and nickel was observed in 11 (85%) and 6 (46%) cases, respectively.

Conclusion: High serum concentration of heavy metals like lead & nickel or high platinum accumulation in seminal cells or high serum estradiol alone or in combinations may be underlying aetiologic factors in human spermiation defect.

Keywords: Lead, Nickel, Oestrogen, Platinum, Sperm release

INTRODUCTION

Spermiation is a process of release of mature elongated sperm into the lumen of seminiferous tubule. During this process several changes occur in spermatid and tubules. These are removal of cytoplasm from spermatid, dissolution of tubulo-bulbar complexes as well as adhesive junction and finally phagocytosis by Sertoli cells of residual bodies [1,2]. Adhesions exist between Sertoli cells & late spermatids and release of spermatid is time bound controlled process [3]. Spermiation is initiated at the beginning of stage VII of spermatogenesis in rat and mouse (corresponds to human stage II) when the majority of late spermatids align along the luminal edge [1].

Diagnosis of spermiation defect is mainly from testicular histology (normal spermatogenesis but no/very few sperms in tubular lumen) and electron microscopy (defect in tubulo-bulbar complexes & ectoplasmic specialization). Spermiation is a sensitive process and easily disrupted. Besides genetic causes, gonadotropin suppression [4], deficiency of vitamin A and reproductive toxicants [5] have been shown to interfere spermiation in animal. In human, several factors causing male infertility are known but whether they have a role in spermiation defect is not known. Sertoli cells display receptors for Follicular Stimulating Hormone (FSH), androgen, vitamin A (retinoic acid), etc and regulate spermiation [6]. The suppression of FSH or androgen alone causes spermiation failure [2,7,8]. Studies on transgenic mouse models have revealed that retinoic acid (vitamin A) is essential for spermiation and acts through RARα/RXRβ receptors of Sertoli cells [9-12]. Spermiation failure is a frequent feature of defective retinoic acid signaling [12]. Animal studies also have shown that oestrogen impairs spermiation [13,14]. Exogenous estradiol administration increases germ cell apoptosis and spermiation failure in mice through suppressing FSH and intra-testicular testosterone and increasing intra-testicular estradiol [13]. Various environmental toxicants and endocrine disruptors also disrupt spermiation [15]. Disruption to spermiation can impact sperm count and thus, can be the underlying cause of azoospermia or oligozoospermia leading to infertility.

Studies on spermiation defect are on animals [2,5,16,17] and none on human due to difficulty in diagnosis as this involves generous testicular histology and electron microscopy. However, a subset of human spermiation defect may possibly suspected/diagnosed indirectly from finding of non-obstructive azoospermia in presence of normal spermatogenesis on testicular Fine Needle Aspiration Cytology (FNAC) and normal reproductive hormones parameter. In order to understand the aetiologic factors of spermiation defect in human, present study was carried out to explore various aetiologic factors like chromosomal, sertoli cell status, oestrogen, vitamin A and heavy metals.

MATERIALS AND METHODS

This prospective pilot study was conducted in the Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi from February 2010 to December 2011. The Institutional Human Ethics Committee approved the study. Written consent was obtained from each patient before inclusion in the study. A total of 13 spermiation defect and 20 normal control males (10 controls for metals) were studied.

Inclusion criteria for the study were long-standing infertility, non-obstructive azoospermia or oligozoospermia, normal secondary sex character, normal genitalia, normal hormones (testosterone, gonadotropins and prolactin) and normal spermatogenesis on testicular FNAC. All patients with possible aetiological factors for azoospermia viz., testicular maldescent, testicular malignancy, treatment with radiotherapy or chemotherapy, vasectomy, congenital absence of the vas deferens, varicocele, mumps orchitis, trauma, orchidectomy and reproductive endocrine abnormality (testosterone, gonadotropins & prolactin) were excluded from the study. Control males were selected from staff and student of the department with normal secondary sex character, semen parameters and FSH as well as testosterone.

The minimum initial evaluation was a complete physical examination, medical history and reproductive hormones. The evaluation process included history of prior fertility, viral orchitis, cryptorchidism, genital trauma, inguino-scrotal surgery, exposure to radiation, chemotherapy, heat, medications or family history of cystic fibrosis. Physical examination included examination of testis (size, site, consistency, etc), secondary sex characteristics (body habitus, hair distribution and gynaecomastia), vasa deferentia (presence/absence, cyst, etc), epididymis (cyst, mass, etc.,) and varicocele. Semen analysis was performed twice at interval of 3 months. Azoospermia was diagnosed after centrifugation (at 3000g) and microscopic examination of seminal deposit. A scrotal ultrasound scanning of the testes was performed to evaluate testicular size and to rule out varicocele, epididymal cyst or any other genital lesions. The initial hormonal evaluation was serum testosterone, prolactin and FSH. FNAC of testes (bilateral) was performed in all cases. Fluorescence In Situ Hybridization (FISH) with XY probes was carried out in addition to conventional chromosome analysis to find out chromosomal abnormalities, in particular sex chromosome aneuploidy & mosaicism. Yq microdeletion status was also investigated by Sequence Tagged Sites Polymerase Chain Reaction (STS PCR) method. The Anti-Mullerian Hormone (AMH), inhibin B and lactate (seminal) were estimated by Enzyme-Linked Immunosorbent Assay (ELISA) in all cases [18].

Hormone assays like FSH, Luteinizing Hormone (LH), prolactin, testosterone and estradiol were measured through Abbot Axym automated system. AMH & inhibin B were estimated through commercially available ELISA kits (AnshLabs, USA). Vitamin A concentration in serum was estimated according to Kubler and Lorenz method [19]. The reference ranges for normal values of the laboratory or control mean value (±SD) were considered for comparison.

Elements (metals, metalloids and non-metals) in the seminal cells/seminal debris were analysed using Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectrometer (EDXS). It relies on interactions between electromagnetic radiation and matter, analysing X-rays emitted by the matter in response to being hit with charged particles [20]. The characterization capability is related on fundamental principle that each element has a unique atomic structure thus allowing X-rays identification uniquely. Each semen sample (1ml) was fixed in glutaraldehyde solution for 2 hours and centrifuged for 5 minutes at 10950g, after which supernatant was discarded and pellet was washed with phosphate buffer saline. Samples were again centrifuged for 5 minutes at 10950g, supernatant was discarded and pellet was subjected to metal coating with carbon /silver /gold /etc. Random areas (at least two) were selected for X-ray exposure, energy released was measured and mean value calculated for comparison with control. Spectrum showing peaks are used for qualitative and quantitative analysis of all elements.

Metals (manganese, nickel, lead, platinum, mercury, arsenic, gold, cadmium, cobalt, chromium, copper, iron, selenium, magnesium and zinc) were also measured in serum using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). ICP multi-element (Ag, Al, B, Ba, Bi, Ca, Do, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Ti, Zn) standard solution was purchased from Merck (Darmstadt, Germany; product no. 1.11355.0100, batch no. HC061563). Mercury (product no. 19795.0500, Batch no. 45318616), arsenic (product no.1.19773.0500, batch no. 45303317) and platinum (product no. 1.70219.0100, batch no. HC086348) standards were also obtained from Merck Chemicals (Darmstadt, Germany). Selenium standard (S-9760, lot no. 15H3423) from Sigma Chemicals (St. Louis, MO, USA) and gold standard (product no. 072058, batch no. 733605) from SRL (Mumbai, India) were purchased separately. Supra-pure nitric acid and hydrogen peroxide were purchased from Merck Chemicals, India. Serum sample digestion was carried out with nitric acid and hydrogen peroxide with microwave assistance. The digested samples were cooled, diluted to 10 ml clear solution. The resulting sample solutions were subjected to filtration before proceeding further. Wave-length was selected from a predefined set for each element using the ICP software (version 5.2, Horiba Jobin Yvon, Longjumeau, France). The precision was established by duplicate run for each sample. For calibration curve, the standard solution mixture was diluted stepwise with 5% nitric acid in the concentration range of 5–100 ppb. Three replicates were prepared for each analyte concentration.

STATISTICAL ANALYSIS

All data were analysed using STATA statistical software (version 11). Descriptive statistics were taken as the mean and SD. Wilcoxon rank-sum (Mann-Whitney) test was used to see the mean significance differences between study and control groups. Non-parametric test was used when standard deviations were very high.

RESULTS

The study was based on 13 cases of possible spermiation defect and 20 (10 for metals) normal control males. All cases had normal testes volume, secondary sex character, baseline hormonal levels (testosterone, gonadotropins and prolactin), azoospermia or oligo-azoospermia (1-2 dead sperms/hpf) and normal spermatogenesis on FNAC.

The mean age of spermiation defect group was 31.69 years with a range of 27 to 42 years. [Table/Fig-1] shows details of investigation and their comparison with controls. There was no significant difference in serum FSH, testosterone, prolactin, lactate, AMH, inhibin B & vitamin A between spermiation defect and control groups. Estradiol was found to be high in 6 out of 13 (46%) cases of spermiation defect. All cases of spermiation defect were normal chromosomally as well as negative for Yq microdeletions.

Differences in serum level of various metals [Table/Fig-2] were observed (higher concentration in study group observed with lead, nickel, cadmium, chromium & manganese and lower concentration in study group was observed with arsenic, platinum and zinc) on spectrometry (ICP-AES). In seminal plasma, significant low value of platinum and iron was detected by spectrometry (ICP-AES) as compared to controls [Table/Fig-3]. The data was also analysed case to case individually and we observed very high value (four times or more of control mean) of lead and nickel [Table/Fig-4] in several cases (11 and 6, respectively) through ICP-AES. High content of platinum was detected in seminal cells in 4 cases (out of 8 samples tested; 50%) on elemental scanning electron microscopy [Table/Fig-4]. Two cases had additional high iron (besides platinum) content in seminal cells.

DISCUSSION

This study has shown that spermiation defect exists in human and seems associated with high oestrogen and heavy metals. Vitamin A deficiency or sertoli cell immaturity/dysfunction was unrelated to human spermiation defect in this study. Although spermiation defect

Parameter	Control (20)	Spermiation Defect (13)	p-value*
Age (years) Mean ± SD (range)	28.25 ± 6.82 (20-37)	31.69 ± 4.5 (27-42)	0.11
FSH(mIU/ml) Mean ± SD (range)	6.92 ± 2.76 (2.49-11.8)	5.65 ± 3.11 (2.22-11.65)	0.22
Prolactin(ng/ml) Mean ± SD (range)	Not done NLRR 3.46-19.40	9.03± 4.91 (4.37-19.8)	NA
Testosterone(ng/ml) Mean ± SD (range)	5.00 ± 2.02 (2.11-8.84)	5.06 ± 2.42 (0.84- 10.9)	0.96
Oestrogen (pg/ml) Mean ± SD (range	24.63± 8.34 (12.2- 41.2)	45.92±15.5 (18-73) High in 6 (46%)	0.01(S)
Inhibin B (pg/ml) Mean ± SD (range)	138.39± 50.32 (28.07-212.45)	145.36±92.69 (39.24-296.5)	0.88
AMH(ng/ml) Mean ± SD (range)	11.61± 5.47 (4.54-24.3)	8.86±7.7 (3.24-29.4)	0.55
Vitamin A (nmol/µl) Mean ± SD (range)	518.1 ± 212.4 (269.46-839.5)	708.1 ± 1507.17 (303.5-4188)	0.65
LactateSemen (nmol/µl) Mean ± SD (range)	66.26± 26.7 (14.2 -105)	54.01±36.4 (3.2 -105)	0.58

[Table/Fig-1]: Showing laboratory tests results of spermiation defect cases and controls.
NLRR: normal laboratory reference range; NA: not applicable
* Mann-Whitney test was used to find out significance

Parameter	Control (10)	Spermiation Defect (13)	p-value*
Metal (ppb)	Mean ± SD (range)	Mean ± SD (range)	
Pb	6.37± 4.94 (0.92-15.21)	39.82 ± 12.6 (19.97-58.6)	0.0001 (S)
Cd	0.23± 0.09 (0.13-0.44)	0.49 ± 0.14 (0.18-0.66)	0.0001 (S)
Ni	1.61 ± 1.18 (0.14-3.18)	6.3 ± 3.7 (1.04-14.17)	0.001 (S)
As	8.16± 0.34 (7.80-8.69)	6.08 ± 1.9 (2.33-8.46)	0.002 (S)
Pt	82.08± 1.67 (79.43-85.78)	77.92 ± 4.01(66.2-82.69)	0.005 (S)
Zn	1207.3±103.6(1042.3-1340.8)	978.8 ± 336.9 (385.5-1380)	0.05 (S)
Mn	1.53±1.28(0.1-3.7)	5.25 ± 2.9 (1.1-13.3)	0.001(S)
Hg	0.53± 0.06(0.45-0.63)	0.48± 0.28 (0.08-0.93)	0.58
Au	57.46± 2.11 (55.15-61.92)	58.98± 6.51 (48.8-71.9)	0.48
Co	1.67± 1.19(0.20-3.96)	1.98± 1.74 (0.33-5.3)	0.63
Cr	2.64± 0.72(1.87-4.25)	4.14± 1.67 (1.25-7.4)	0.015(S)
Cu	1396 ± 337.14 (873.9-2143.4)	1385.9± 305 (681-1909.5)	0.94
Fe	1524.4±910.5 (435.6-3215.5)	1244.3± 427.4 (564-1846)	0.33
Se	85.59± 13.02 (48.65-91.81)	81.75± 7.10 (67.6-92.59)	0.26
Mg	18.28± 10.16 (11.40-39.85)	25.88± 17.7 (3.3-59.23)	0.24

[Table/Fig-2]: Showing heavy metal laboratory tests results of spermiation defect cases and controls in serum.
* Mann-Whitney test was used to find out significance. (S)-significance ≤ 0.005

is diagnosed by testicular histology (normal spermatogenesis but no/very few sperms in tubular lumen) and electron microscopy (defect in tubulo-bulbar complexes & ectoplasmic specialization) in animal models, these are difficult to carry out in human. Hence, we have included cases as spermiation defect when patient presented with non-obstructive azoospermia/oligzoospermia with normal sexual development, secondary sex characters, gonadotropins, gonadal hormones and testicular histology/cytology.

In this study of 13 cases of spermiation defect we have detected possible association with serum high estradiol (6 cases/46%), seminal cells high platinum (4 cases/50%) and serum level of high metals (four times or more), in particular lead (11 out of 13 cases/85%) and nickel (6 out of 13 cases/46%). Other metals like manganese (higher serum value) or zinc, arsenic and mercury (lower serum value; at least three times less) were also observed [Table/Fig-4]. We did not find any detectable cause/associations in one case ([Table/Fig-4]; case 10). However, we cannot comment on role of adhesion molecules, adherens junction dynamics, ectoplasmic specialization dynamics, etc (laminin, cadherin, catenin, etc) with human spermiation defect as we did not investigate for these aspects in this study although animal experiments suggest their role in spermiation defect [21-23]. LKB1 or STK11 (serine/threonine kinase 11) is a protein kinase regulator and its reduced expression in testis leads to spermiation defect and infertility in mice due to defects in ectoplasmic specializations [23]. These areas need to be explored in coming years.

We have found both high estradiol and metals in five cases. We did not find any association with chromosomal abnormality or Yq microdeletion. Sertoli cell plays an important role in spermiation (reference) however we did not find any Sertoli cell problem (maturation/ number/ function) as evident by normal AMH and inhibin B levels. AMH was normal and no case with high AMH was observed, as would be expected in Sertoli cell immaturity or hyperplasia/ tumour. Similarly, normal level of serum concentration of inhibin B reflects normal functional interaction between germ cells and Sertoli cells [24,25].

Role of vitamin A in normal spermatogenesis and spermiation is well documented [26]. Prolonged vitamin A deficiency may results in spermatogenic arrest, germ cell loss and spermiation failure [27]. Contrary to previous animal studies, our study showed normal levels of vitamin A in 12 cases of spermiation defect, although number of cases studied was small. In one case we observed very high vitamin A level in contrary to low value as expected with spermiation defect.

High serum oestrogen also causes spermiation defect [14]. This study detected high serum estradiol in 6 cases (46%) and we think this may be responsible/partly responsible for failure in sperm release. The presence of oestrogen receptor beta and aromatase in the germ cell has highlighted the physiological role of oestrogen in spermatogenesis. Oestrogen receptor alpha knockouts and aromatase knockout mice have further accentuated the role of oestrogen in germ cell maturation & spermiation. In rat, high dose of oestrogen decreases intratesticular testosterone and disrupts spermiation [13]. Estradiol has been shown to cause stage-specific changes in sertoli cell microtubules, which may underlie the reason for failure to initiate spermiation. Our findings also support role of high serum estradiol with spermiation defect.

Role of heavy metals with human spermiation defect is not available in literature. However, an association of heavy metals with dark-coloured semen from primary testicular failure was reported earlier [20]. Heavy metals are known to affect testicular function including damage to blood testes barrier or tight junction [28]. Lead toxicity in human alters testicular histology [29]. Nickel also has been shown to interfere with the reproductive capacity of male rats [30,31]. Cadmium is known to damage Sertoli tight junctions that constitute blood testes barrier [32,33]. Chromium exposure results

Parameter	Control (10)	Spermiation Defect (13)	p-value*
Metals (ppb)	Mean ± SD (range)	Mean ± SD (range)	
Pb	52.46±85.83 (1.48-233.3)	34.69±13.57 (12.44-54)	0.46
Cd	0.32±0.41 (0.07-1.48)	0.47±0.08(0.36-0.65)	0.2
Ni	5.35±10.54 (0.05-32.87)	5.41±1.42 (1.8-7.1)	0.98
As	6.25±0.75 (4.79-7.29)	5.58±1.06 (4.0-7.3)	0.12
Pt	87.08±12.3 (80.37-121.81)	76.86±3.31 (68.98-81.8)	0.008 (S)
Zn	1248.87±610.16 (464-2109)	1456.8±702.3 (793-3198.8)	0.46
Mn	2.38±1.78 (0.3-4.8)	2.97±1.71 (0.6-5.8)	0.42
Hg	0.26±0.1 (0.07-0.43)	0.24±0.12 (0.03-0.41)	0.67
Au	62.25± 8.25(58.52-84.67)	58.84± 2.96 (50.0-63.1)	0.08
Co	0.15± 0.26 (0.01-0.87)	0.10± 0.10 (0.01-0.42)	0.53
Cr	2.91± 3.27(0.24-10.0)	3.22± 1.80 (0.10-5.5)	0.77
Cu	1362.05± 492.7 (136.9-1823.1)	1262.9± 325.86 (710.5-1681.3)	0.56
Fe	1365.2 ± 237.8 (1123.6-1911.7)	910.64± 413.7 (125.3-1247.5)	0.005 (S)
Se	82.23± 2.93 (77.05-86.33)	80.89± 4.44 (72.5-87.16)	0.41
Mg	19.54± 15.78 (4.20-48.7)	26.33± 19.07 (10.1-64.9)	0.37

[Table/Fig-3]: Showing heavy metal laboratory tests results of spermiation defect cases and controls in seminal plasma.
* Mann-Whitney test was used to find out significance

ICP-AES	Case 1	Case 2	Case 3	Case4	Case 5	Case 6	Case 7	Case 8	Case 9
Metals (ppb)	DB209	DB208	DB202	DB10	DB215	DB210	DB211	DB201	DB179
Mn	5.5	4.6	5.8	1.1	6	5	7.5	2.5	13.3
Ni	5	8.6	1.04	4.4	8.8	9.5	10.2	3.84	14.17
Pb	25.3	40.3	19.97	28.2	40.4	48.6	47.3	32.03	51.25
Pt	78.9	77.3	82.69	77.4	74.8	76.2	79.6	78.93	81.49
Hg	0.42	0.21	0.59	0.59	0.11	0.08	0.29	0.56	0.25
As	7.31	6.13	8.39	8.3	5.3	5.2	4.2	8.47	2.33
Au	60.5	57.6	57.72	57.6	57.8	58.8	57.2	60.43	58.67
Cd	0.27	0.66	0.18	0.47	0.64	0.63	0.48	0.47	0.49
Co	1.2	0.9	1.36	5.3	0.8	0.56	0.33	1.25	1.52
Cr	4.9	5.1	2.32	3.4	6	5.4	4.1	5.48	4.1
Cu	1198	681	1457.5	1909.5	1487.7	1579	1206	1623.4	1313.5
Fe	1511	1192	1471.8	573	1101	842	564	1441	1592.9
Se	84.5	82.5	92.59	89.4	75.9	77	75.1	91.11	86.49
Mg	34.96	21.65	14.8	34.12	3.3	13.3	5.14	57.53	30.82
Zn	1220	902.9	1268	1237.7	518.6	410	385.5	1380.1	1064.8
EM									
Platinum Wt%	0	1.47	0	1.75	not tested	not tested	not tested	0	2.75
Platinum Atomic %	0	0.11	0	0.18	not tested	not tested	not tested	0	0.2
E2	61	42	59	44	55	57	39	47	21
Remarks	Pb, E2	Pb,Ni,Pt	E2	Pb, Pt	Pb,Ni, Hg,Mg,E2	Pb,Ni,Hg,Zn,E2	Pb,Ni, Mn,Co,Mg,Zn	Pb,E2	Pb,Ni, Mn,Ar, Pt

ICP-AES	Case 10	Case 11	Case 12	Case 13	Control	Control	Control	Remarks
Metals (ppb)	DB94	DB214	DB213	DB204	mean	range	SD	
Mn	3.9	4.1	3.5	5.5	1.53	0.1-3.7	1.28	more than 4 times in 2
Ni	3.19	3.57	3.04	7.5	1.61	0.14-3.18	1.18	more than 4 times in 6
Pb	23.92	49.87	52.03	58.6	6.37	0.92-15.21	4.94	more than 4 times in 11
Pt	76.67	79.87	79.91	66.2	82.08	79.43-85.78	1.67	
Hg	0.93	0.62	0.76	0.88	0.52	0.45-0.63	0.06	less than one third in 2
As	7.12	7.09	5.17	4.2	8.16	7.80-8.69	0.34	less than one third in 1
Au	42.29	71.91	70.48	48.8	57.46	55.15-61.92	2.11	
Cd	0.52	0.48	0.57	0.6	0.23	0.13 - 0.44	0.09	
Co	4.55	4.55	3	0.46	1.67	0.20-3.96	1.19	<one third in 2; >4X in 1
Cr	7.41	2.98	2.48	4.4	2.64	1.87-4.25	0.72	
Cu	1271.2	1190.6	1620.4	1379	1396	873.9 - 2143.4	337.14	
Fe	1846.5	1258.9	1841	942	1524.4	435.6-3215.5	910.5	
Se	80.67	78.9	81.11	67.6	85.59	48.65-91.81	13.02	
Mg	19.51	29.98	59.23	12.3	18.28	11.40-39.85	10.16	less than one third in 2
Zn	1146.6	1100	1180.4	910	1207.3	1042.3-1340.8	103.6	less than one third in 2
EM								
Platinum Wt%	0	not tested	not tested	3.67	0	0	0	
Platinum Atomic %	0	not tested	not tested	0.26	0	0	0	
E2	18	36	44	73	24.7	18-73	9.37	11 to 44 (lab range)
Remarks	no cause	Pb	Pb	Pb, Ni, Co, Pt, E2				

[Table/Fig-4]: * Showing case wise details of heavy metal and oestrogen levels.
EM: electron microscopy; E2: estradiol

in abnormal spermatogenesis, prematurely released spermatocytes and spermatids in animals [34]. In men, occupational exposure to chromium has been shown to be associated with abnormal semen quality [35]. Cobalt-chromium toxicity has been shown to decrease sperm motility, viability and concentration and increased testicular damage via oxidative stress [36]. Manganese exposed workers have been shown to have reduced sperm motility and concentration [37]. We have found very high serum level of various metals (four times or more from control mean; lead, nickel and manganese) or very low level (three times or less from control mean; arsenic, mercury, magnesium, zinc and cobalt) in serum with spermiation defect in our series. We did not find any specific occupation (heterogenous occupation) or geographic base (no two cases from same area) for high serum lead/nickel. We have also observed significantly lower value of platinum in both serum as well as seminal plasma in spermiation defect. In contrary, we have observed very high concentration of platinum in seminal cells in 50% of cases (4 cases out of 8 cases). These contradictory findings on platinum could be due to selective cellular accumulation of platinum or differences resulted following frozen serum/seminal plasma (for spectrometry) vs. fresh seminal cells (for electron microscopy) as test materials. Platinum derivatives have shown to cause degenerative changes including disruption of tight junctions in Sertoli cells followed by abnormal mitosis and malformation of germ cells [38]. Iron is transported to germ cells as a nutritional component via Sertoli cells with the help of transferrin. High level of iron in seminal cells might be due to failure of transfer to germ cells interfering in germ cell development as shown by a study where mutant mice lacking normal transferrin revealed a decreased number of germ cells and highly reduced spermiation [39].

Heavy metal toxicity impairs antioxidant status of organs thus making organs vulnerable to oxidative stress. Oxidative stress also causes mitochondrial dysfunction thus defects in spermiation [40]. Mitochondria produce more superoxide anions under oxidative stress which initiate intrinsic apoptotic pathway. Oxidative stress in aging testes impairs spermiation [41]. Heavy metal induced

oxidative stress may be a major factor contributing to spermiation defect in our study. We have observed several cases with high metals (serum/seminal cells) and oestrogen or either high metal/s or high estradiol ([Table/Fig-4]; case 3). This finding, although derived from only 13 cases, indicates altered (very high/low) serum/seminal cell concentration of heavy metals as well as oestrogen alone or in combinations are important aetiologic factors for human spermiation defect.

LIMITATION

For practical, technical, ethical and logistical reasons, we could not confirm diagnosis (through open testicular biopsy & electron microscopy) and obtain sufficient number of cases for the study.

CONCLUSION

Spermiation defect exists in human and seems associated with high oestrogen and heavy metals (lead, nickel, platinum, etc.,). Vitamin A deficiency or sertoli cell immaturity/dysfunction was unrelated to human spermiation defect. The mechanisms by which heavy metal induce spermiation defect, whether by oxidative stress or any other pathways, are future areas of research. As the number of cases are few the study needs to be tested with larger number of spermiation defect cases.

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