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Semen Quality of Industrial Workers Occupationally Exposed to Chromium

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Abstract: Semen Quality of Industrial Workers Occupationally Exposed to Chromium: Sunil KUMAR, *et al*. Reproductive Toxicology and Histochemistry Division, National Institute of Occupational Health (ICMR), India—A total of sixty-one subjects occupationally exposed to chromium in an industry which manufactures chromium sulphate and fifteen control subjects from a nearby industry which does not manufacture any chromium related compounds were studied. The history of each subject was recorded on pre-designed form through interview and a routine medical examination was carried out. Blood samples (5–6 m*l*) were collected for the estimation of chromium and semen samples were collected for semen analysis and the determination of copper and zinc levels in the seminal plasma. Clinical examination revealed nasal perforation in 10 subjects (out of 61) in the exposed group as compared to none in the control group. A significantly higher level of chromium was observed in the blood of the exposed workers as compared to the control. The concentration of zinc in seminal plasma was lower while the level of copper was higher in the exposed group as compared to the control. However, these changes were not statistically significant. Statistically significant higher numbers of morphologically abnormal sperms were noticed in the exposed group with respect to the control. Further analysis of the data indicated that about 53% of the exposed subjects showed less than 30% normal forms as compared to 10% in control subjects. However, no significant alterations in semen volume, liquefaction time, mean pH value, sperm viability, concentration or motility, were noticed between chromium exposed and unexposed workers. The data also indicates that exposure to chromium has some effect on human sperm as a significant positive correlation (r=0.301) was observed between percentages of abnormal sperm morphology and blood chromium levels (p=0.016) after pooling all the data of the control and exposure groups. *(J Occup Health 2005; 47: 424–430)*

Key words: Chromium, Semen characteristic, Sperm morphology, Metal toxicity, Zinc, Copper, Sperm motility, Sperm viability

Chromium is a naturally occurring element found in rocks, plants, animals, soil, volcanic dust and gases. In humans and animals trivalent chromium is an essential nutrient that plays an important role in glucose, fat and protein metabolism by potentiating the action of insulin[1]. Chrome ore mining and chrome based industries also release chromium into the environment. Human beings may be exposed to high doses of chromium in certain occupational and environmental situations. Occupational exposure has been shown to give rise to elevated levels of chromium in blood, urine and some body tissues, inhalation being the main route for chromium exposure[2].

Industrial workers exposed to high doses of chromium compounds may develop asthma, nasal perforation and respiratory diseases. Exposure to hexavalent chromium (Cr VI) is reported to cause pulmonary carcinoma, dermatitis, hepatotoxicity, nephrotoxicity and gastrotoxicity in human and laboratory animals[3]. The experimental study of Saxena *et al*.[4] suggested a risk to growing testes, if rats are exposed to hexavalent chromium during the prepubertal stages of their development, which in turn, may disturb normal testicular physiology in adulthood. Other studies carried out in rats have also demonstrated that chromium causes testicular atrophy and reduces sperm count and motility[5, 6]. However, Glaser *et al*. reported that the histological examination of the testis of rats exposed to 0.2 mg chromium (VI)/m^3 as sodium dichromate for 28 or 90 days through inhalation[7] or 0.1 mg chromium (VI)/m^3 as sodium dichromate for 18 months[8] or 0.1 mg

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chromium/m^3 as a 3:2 mixture of chromium (VI) trioxide and chromium (III) oxide for 18 months[9] revealed no abnormalities. Furthermore, the presence of chromium in fetuses and infants of women working or living near dichromate industries has been reported[10]. Clarkson *et al*.[11] in 1985 found no report in the literature about the effect of any chromium compound on reproduction or prenatal development in humans. At present no substantial information is available regarding the harmful effects of any form of chromium in human reproduction including semen quality or birth defects except for the recent reports of Li *et al*.[12] and Danadevi *et al*.[13] on human sperm among workers exposed in an electroplating factory and welding plant, respectively. Recently Kumar[14] reviewed the data on occupational exposure and reproductive dysfunction and mentioned that detailed studies on human reproduction with reference to heavy metals are scarce, except for lead. The scarcity of data on chromium exposure effects on human sperm even though there are a number of positive reports on chromium induced testicular toxicity in animals and the known affinity of chromium for the testis and incorporation into sperm[15], prompted us to take up this study. In addition, zinc and copper were also measured in seminal plasma as zinc is considered to play an important role in the oxidant defense system[16].

Material and Methods

Subjects

Sixty-one industrial male workers, mean age 33.26 ± 6.97 yr, working in a chromate factory in India which manufactures chromium sulphate were enrolled for this study. A total of 15 workers with a mean age of 24.2 ± 4.26 yr were selected from a pharmaceutical factory, which is about 1 km away from the chromate factory and does not manufacture any chromium related compounds. The characteristics of exposed and control subjects indicated that there were some differences in personal characteristics such as smoking (about 60 and 20% in the exposed and control groups respectively), and drinking (41% drink alcohol in the exposed group as compared to none in the control). However, chewing of betel nut and tobacco was approximately 53 and 47% in the control and exposed groups, respectively. We could not enroll higher numbers of perfectly matched control cases, as the pharmaceuticl workers were not persuaded to take part in the semen analysis. Therefore, data were also compared with WHO reference values[17, 18]. Written consent was taken from each worker after explaining the aim and objectives of the study and only those workers were enrolled who agreed to the objectives of the study. The semen and blood samples were collected during the same time period from all the subjects. A detailed form was filled up by interview technique of individual subjects' social and medical histories including reproductive history. A detailed medical examination was also carried out for each worker. Blood samples (about 5–6 m*l*) were drawn by vein puncture from all the subjects and a portion of the same was used for the chromium determination.

Semen analysis: Each worker was requested to provide a semen sample by masturbation for the assessment of the semen characteristics as well as determination of the concentration of chromium in the seminal plasma. The semen volume was measured to the nearest 0.1 m*l*. The pH of the semen was determined within one hour of ejaculation by putting a drop of semen on a pH paper strip. After about 30–60 s, the colour of the impregnated zone was compared with the colour of the strip having a pH 6 to 9 scale. The liquefaction time of the semen was noted in minutes after the semen collection. Sperm motility was assessed in a drop of semen kept on clean micro-slides using light microscopy within 30–60 min after the collection of the sample and was grouped as rapid+progressive, sluggish or immotile. Viability was determined by using 1% trypan blue solution. Live sperm exclude the dye and remain unstained while dead sperm absorb the dye and are stained. Sperm concentration was determined by using a haemocytometer. For this purpose 20 μl of semen samples were taken using a positive pressure pipette and diluted by sperm diluents. The sperm counts were made in the five central squares (centre and the four corners of the inner square). The slides for the assessment of sperm morphology were prepared on clean micro-slides and stained with Spermac stain (Stain Enterprises Inc, USA) as per the makers' procedure. Minimums of 50 to 200 sperms from each subject were analyzed for sperm morphology using a light microscope (ZENAVAL, Germany), depending upon the availability of well-stained sperm, and expressed in percentage. In most of the cases a minimum 100 sperms were analyzed.

Metal analysis: The seminal plasma was separated by centrifugation of semen at 2000 RPM and kept in a deep freeze –80°C till analysis of zinc and copper metals by an atomic absorption spectrophotometer (Perkin - Elmer Model 3100, USA).

Statistical analysis: Statistical analysis was carried out using Students' 't' and Anova tests in order to note the significance between the two groups as well as to find out correlations if any between various parameters. The values are given as Mean ± SD and the results are presented in Tables 1–4 and Fig. 1. The multiple regression analysis for the pooled data with sperm morphological abnormality (%) as dependent variable and blood chromium level, smoker (0, if non-smoker and 1 otherwise) and alcohol drinker (0 if non-drinker or 1 otherwise) as independent variables was carried out. The regression coefficients for smoker and alcohol drinker were not significant statistically, indicating no effect on morphological abnormality in the present data. Therefore, they were dropped from the regression analysis.

Results

The medical examination of the workers revealed that 16.4% of the subjects showed nasal perforation in the exposed group. However, no nasal perforation was observed in any of the subjects from the control group.

Metal levels: Table 1 summarizes the data on chromium, zinc and copper levels. The chromium level in the blood was significantly higher ($p<0.005$) in the exposed group as compared to the control. It was about three times higher in the blood of exposed subjects as compared to controls. The mean seminal zinc and copper levels in the chromium exposed group showed statistically nonsignificant alteration with respect to the control.

Data of seminal pH, liquefaction time and volume are given in Table 1. The data for seminal pH indicates that a considerable number of subjects showed a higher pH value than the normal range in both groups. No significant alteration in the mean pH value was observed between the two groups. The liquefaction time, semen volume and sperm concentration also did not show any statistically significant differences between the two groups. The data on viability indicates that 6 and 1 subjects showed lower viability, i.e. <50% in the exposed and control groups, respectively. Motility (<50%) was also lower in 3 exposed subjects as compared to none in control group. No statistically significant alteration in mean percentage value of motility and viability was observed between the control and exposed groups (Table 3).

The sperm morphology data revealed deterioration in sperm morphology in the exposed group as compared to control group. The mean morphologically normal sperm was significantly lower in the chromium-exposed workers with respect to that of the control. Furthermore, analysis of sperm morphology data revealed that about 53% of exposed subjects showed <30% normal forms as compared to 10% of control subjects (Table 4). In addition to this, about 13% of cases showed <15% of normal sperm morphology in exposed subjects as compared to none in the control group. Normal sperm morphology was lowest, i.e. 23%, in exposed subjects with nasal perforation as compared to 28.9% in chromium exposed subjects. Furthermore, after pooling all the data together, a statistically significant positive correlation (r=0.301) was observed between the percentages of abnormal sperm morphology and blood chromium levels (p=0.016) (Fig. 1) indicating that increasing chromium levels are associated with increasing morphological abnormality.

Discussion

The clinical examination indicated a number of cases with nasal perforation among the chromium-exposed

Table 1. Chromium concentration in blood and concentrations of zinc and copper in seminal plasma ($\mu g/l$)

Groups	Blood Chromium level ($\mu g/l$)	Seminal plasma zinc level (mg/*l*)	Seminal plasma copper level ($\mu g/l$)
Control	22.8 ± 17.7	155.91 ± 103.4	167.3 ± 151.2
[15]	[15]	[14]	[14]
Exposed	63.7 ± 50.4**	134.53 ± 101.5	196.1 ± 161.8
[61]	[61]	[54]	[54]

Figures in parentheses indicate the number of subjects, ** Significantly higher ($p< 0.005$)

Table 2. Semen volume (ml), liquefaction time (min) and seminal pH level of chromium exposed and unexposed workers

Sr.No.	Parameters	Control	Exposed
1	Semen volume (m*l*)	2.54 ± 0.641	2.67 ± 0.964
		[12]	[55]
2	Liquefaction time (min.)	30.62 ± 5.62	28.03 ± 4.13
		[8]	[33]
3	Mean seminal pH level	7.98 ± 0.27 [12]	7.91 ± 0.36 [51]
	pH Range		
	a) < 7 pH	–	(1.6 %) [1]
	b) ≤7–8 pH	50.0% [6]	58.8% [30]
	c) < 8 pH	50.0% [6]	39.2% [20]

Figures in parentheses [ ] indicate the number of subjects

Table 3. Sperm concentration (million/m*l*), motility, viability and morphology (%) of chromium exposed and control workers

Sr.No.	Parameters	Control	Exposed
1	Sperm Concentration		
	(Mean ± SD)	43.75 ± 29.9	49.57 ± 36.3
		[15]	[61]
	>20 million/m*l*	20%	16.4%
		[3]	[10]
	≤20 million/m*l*	80%	83.6%
		[12]	[51]
2	Sperm Viability (%)		
	Vitality Mean ± SD	60.00 ± 8.68	59.51 ± 11.33
		[14]	[59]
	>50	7.1%	10.17%
		[1]	[6]
	≤50	92.9%	89.83%
		[13]	[53]
3	Sperm Motility (%)		
	Motility Mean ± SD	76.89 ± 5.76	73.77 ± 11.79
		[14]	[59]
	>50	–	(5.08%)
			[3]
	≤50	(100%)	(94 .9%)
		[14]	[56]
	Motility category		
	Rapid /Progressive	50.83 ± 13.59	51.79 ± 10.96
	Sluggish	24.16 ± 8.81	23.25 ± 5.94
	Immotile	23.00 ± 5.76	24.74 ± 9.16
4	Sperm Morphology		
	Normal morphology Mean ± SD	45.10 ± 13.4	27.87 ± 2.5**
		[10]	[54]
	<30% normal	(10%)	(53.7%)*
		[1]	[29]
	<15% normal	–	(12.96%)
			[7]

*Statistically significant using Fisher's exact test p=0.015, **Statistically significant (p< 0.005) using Student's 't' test, Figures in [ ] indicate the number of subjects and figures in ( ) indicate percentages of subjects

Table. 4. Normal sperm morphology (% ± SD) among subjects with or without nasal perforation

Sr. no	Groups	Normal sperm morphology
1	Control (10)	45.10 ± 13.40
2	Exposed (54)	27.87** ± 12.56
3	Exposed subjects without nasal perforation (44)	28.97* ± 13.48
4	Exposed subjects with nasal perforation (10)	23.00* ± 5.33

* p< 0.01, ** p< 0.005 using Fisher Behrens 't' test for unequal variances

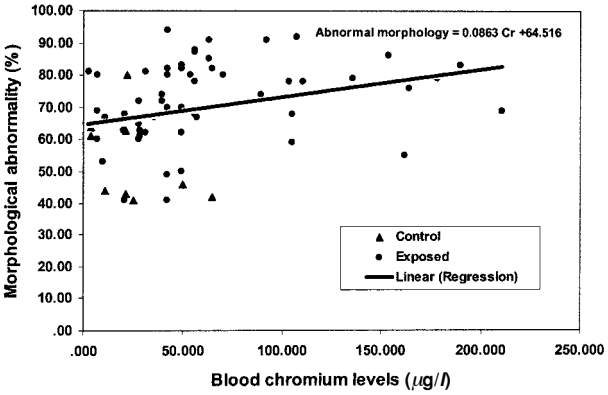

Fig. 1. Relation between blood chromium levels and morphological abnormalities.

workers as compared to none in unexposed workers. This finding corroborates evidence in an earlier report by the International Chromium Development Association, which indicated that chronic effects in humans following occupational exposure to Cr (VI) include perforation or ulceration of nasal mucosa etc.[19] In the present study, deterioration of sperm morphology was more evident among subjects with nasal perforation with respect to both the control and exposed workers without nasal perforation.

In the present study, the blood chromium level was significantly higher in the exposed group with respect to the control. Danadevi *et al.*[13] also found a significantly higher blood chromium level among workers occupationally exposed to chromium in a welding plant. However, Li *et al.*[12] reported insignificant differences between chromium levels in both seminal plasma and serum with respect to controls. Earlier, in an experimental study, about seven times higher chromium levels in the blood of exposed animals were observed as compared to control animals[20]. In addtion, a significant accumulation of total chromium in the blood and testis was also reported among rats exposed to hexavalent chromium[4]. Furthermore, Danielsson *et al.*[21] reported that chromium (especially trivalent chromium) strongly accumulated in the interstitial tissues of gonads of male mice, but not in the seminiferous epithelium.

The zinc level in seminal plasma was lower (statistically insignificant) as compared to the control. However, a significantly lower level of zinc in the seminal plasma of chromium exposed workers was observed by Li *et al.*[12] They also reported that further studies are required to understand the effect of zinc on semen status. It is likely that a decrease in zinc concentration in seminal plasma may reduce the overall anti-oxidant level against Cr (VI)- induced ROS, resulting in oxidative injuries to germ cells. Furthermore, Reeves and Rossow[22] also reported that zinc deficiency leads to impairment of the maturation of spermatids. In a recent study at our laboratory, a lower level of zinc was observed in azoo and oligospermic subjects with respect to normospermic subjects (unpublished).

The present study clearly shows that chromium exposure leads to significant alteration in sperm morphology. The experimental study of Saxena *et al.*[4] showed that rats exposed from weaning to 90 d with 1,2 and 3 mg/kg i.p. of hexavalent chromium had a significant reduction in sperm counts, and also motility in the higher dose of chromium treatment group. However, no increase in sperm malformation was seen in any of the treatment groups as compared to the control. Another experimental study using rats indicated that chromium treatment over a period of 90 d caused partial loss of cellular activity in testicular tissues of rats whereas treatment with high doses of chromium caused deleterious effects both on spermatogenic and steroidogenic activity[23]. A reduction in sperm count and degeneration of the outer layer of the seminiferous tubules was seen in male mice fed with 15.1 mg chromium (VI) /kg/d potassium dichromate for 7 wk reported by Zahid *et al.*[24] However, NTP data on potassium dichromate feeding studies in mice and rats did not show such effects after exposure to 32.2 mg chromium (VI)/kg/d in mice and 8.4 mg chromium (VI)/kg/d in rats[25, 26]. Elbetieha and Al-Hamood[27], noted that ingestion of trivalent and hexavalent chromium compounds by adult male and female mice could also cause adverse effects on fertility and reproduction.

Welders constitute a major occupational group with acknowledged exposure to chromium[28]. Recently Kumar *et al.*[29] reported that welding might have some adverse effects on sperm motility, morphology and physiologic functions even though the sperm concentration was in the normal range. Recently, Danadevi *et al.*[13] reported deterioration in sperm quality among welders exposed to nickel and chromium. They reported a significant positive correlation between the percentages of tail defects and blood nickel concentrations in exposed workers and a negative correlation with sperm concentrations and blood chromium levels. However, in another study conducted by Bonde and Ernest[30] among tungsten inert gas stainless steel welders and mild steel welders showed no deterioration with increasing levels of internal exposure to chromium. Recently, Li *et al.*[12] observed a significant reduction in the sperm count, motility and zinc levels among chromium exposed workers as compared to controls[17]. Serum FSH level were also significantly higher among exposed workers than the control. However, no significant differences were observed in semen volume, semen liquefaction time, and serum LH level. On the basis of clinical and experimental studies they suggested that occupational exposure to chromium (VI) leads to alteration of semen status and may affect the reproductive success of exposed workers.

At this moment it is difficult to speculate about the mechanism of the observed changes in sperm morphology even though other parameters studied were within normal ranges. Further studies are needed to elucidate the mechanism of the deterioration in sperm morphology. Murthy *et al*.[31] studied the ultrastructural changes in testicular tissue of chromium treated rats and reported a significant increase in the blood and testicular chromium levels. Although no light microscopic pathological changes in epididymal sperm counts and motility were observed, under EM they observed that late stage spermatids were the most affected germ cells. The observed alterations in sperm morphology in this study may result in the disruption of normal testicular physiology leading to reproductive impairment after chromium exposure. The present study didn't find any alteration in sperm concentration, viability and motility in relation to chromium exposure, however a significant association between deterioration in normal sperm morphology and blood chromium level was observed. Furthermore, higher sperm morphology defects were noticed in the subjects with nasal perforation with respect to the control.

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