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Semen quality of Indian welders occupationally exposed to nickel and chromium

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Abstract

The semen quality of 57 workers from a welding plant in South India and 57 controls was monitored. Blood nickel and chromium concentrations were determined by ICP-MS. Analysis of semen samples was performed in accordance with World Health Organization criteria. The blood level of nickel and chromium for the 28 exposed workers was 123.3 ± 35.2 and 131.0 ± 52.6 μg/l, resepctively, which was significantly higher than the 16.7 ± 5.8 and 17.4 ± 8.9 μg/l for the control group ($n = 27$). Sperm concentrations of exposed workers were 14.5 ± 24.0 millions/ml and those of the control group were 62.8 ± 43.7 millions/ml. Rapid linear sperm motility was decreased in exposed workers compared to controls. There was a significant positive correlation between the percentage of tail defects and blood nickel concentration in exposed workers. The sperm concentration showed a negative correlation with blood chromium content in workers. More abnormal characteristics were found in the semen of exposed workers. Semen abnormalities correlated with the number of years of exposure to welding fumes containing nickel and chromium.

Keywords: Welders; Sperm quality; Occupational exposure; Nickel; Chromium; ICP-MS

1. Introduction

There is growing concern about the increasing prevalence of various abnormalities of the reproductive system in human males. The reasons for this occurrence could be increased stress, lifestyle factors, and presence of endocrine-altering chemicals in the environment. Occupational activities may involve constant exposure to toxic agents and may have a detrimental effect on human reproduction [1]. Further, human males are of relatively low fertility as compared to other mammals and hence may be at a greater risk from reproductive toxicants. For example, the number of sperm per human ejaculate is typically only 2- to 4-fold higher than the number at which fertility is significantly reduced, whereas the number of sperm in rat, rabbit, or bull ejaculates is many times (up to 1400-fold) the number that will produce maximum fertility. Human males have markedly smaller relative testis size and the lowest rate of daily sperm production per gram of testes (by a factor of more than 3) as compared to the mouse, rat, or monkey. The percentages of progressively motile sperm and morphologically normal sperm in human semen are also lower than in any of the animal model [2].

Several epidemiologic studies have shown a correlation between paternal occupation and incidence of childhood cancer. A significantly increased risk of cancer has been observed among offspring of fathers employed in jobs involving exposure to metals [3]. Anthropogenic use has led to global dispersion of metals in the environment. Because of their wide distribution and extensive use in modern society, some human exposure to toxic metals is inevitable. Hence, as a class of agents, toxic metals are a concern of the highest priority for human exposure.

The heavy metals nickel and chromium are widely distributed in the work place. Nickel has many industrial and commercial uses. It is extensively used in the plating industry, sometimes in combination with other metals. Its other uses are in electroplating, welding, flame cutting, flame spraying, and mold making. Nickel is also used in the manufacture of jewelry, coinage, cutlery, cooking utensils, and dental or surgical prostheses [4]. Chromium is widely used in metallurgy, chrome plating, welding, the chemical industry, textile manufacture, wood preservation, photography

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and photoengraving, the refractory industry, and cooling system [5].

Nickel compounds are well-established human carcinogens [6], though the precise molecular mechanism of carcinogenesis is undefined. Nickel crosses the placental barrier, directly affecting the developing embryo or fetus in experimental animals [7]. Spermatotoxic effects of nickel in mice have also been observed [8]. Chromium is known to cause allergic dermatitis, adverse tissue damage, and carcinogenic effects in mammals [9]. The adverse effects of chromium due to occupational exposure on the central nervous and reproductive systems, as well as genotoxicity and carcinogenicity in workers, have been documented. A number of epidemiologic studies have shown a strong association of occupational exposure to chromate particles in welders and electroplating workers and the incidence of nasal and lung cancer [10].

Relatively few data are available regarding the possible reproductive effects of metals in men [11,12] except lead, which has been thoroughly studied [13,14]. Moreover, conflicting results have been reported on the subject [15,16]. Studies on the reproductive effects in occupationally exposed workers of nickel and chromium are scanty [17–19], and no investigation from India has been reported.

In men, the more accessible reproductive end product is the semen. Reproductive toxicants may have an adverse effect on the male reproductive system by directly affecting the process of spermatogenesis leading to reduced semen quality [20]. Semen analysis is regarded as a valid approach for assessing male reproduction. In order to examine potentially hazardous workplace exposure, semen quantity and quality have often been used as a marker of male reproductive function [21].

The objective of the present study was to analyze the semen of welders exposed to nickel and chromium in South India. In addition, blood nickel and chromium levels were quantified using the ultra mass 700 inductively coupled mass spectrometer (ICP-MS). In recent years, ICP-MS has emerged as the benchmark technique in laboratories worldwide and offers very high sensitivity. Its detection power enables the precise determination of metallic contents in a variety of samples. This technique also requires little sample preparation even when the elements are present in the ng/g range.

2. Materials and methods

2.1. Subjects

The study group was employed at a welding plant located in south India. This group was composed of 57 workers who had been exposed for 2–21 years to welding fumes. The control group consisting of 57 subjects was matched for age, lifestyle, and economic status. The control subjects were not exposed to known harmful chemicals. A detailed questionnaire was completed for each of the 114 subjects prior to the start of the study. The questionnaire was intended to elicit information on the subject's age, smoking habits, duration of exposure, and medicine usage. Medical histories were obtained and physical examinations were performed, with special emphasis on the genitourinary tract. The medical history included questions about the subject's reproductive history. The possibility of exposure to other gonadotoxic agents and the presence of some addictions (e.g. smoking, alcohol abuse) also were explored. The institutional ethical committee approved the research procedures utilized in this study. All subjects were informed of the objective of the study and gave their consent.

2.2. Analysis of nickel and chromium content

Twenty-eight male welders and 27 control men were selected randomly from the total number of subjects for blood sampling for metal analysis. Blood was sampled during the morning hours on Thursday (the 4th day of the workweek). For estimating nickel and chromium content, the ultra mass 700 inductively coupled plasma mass spectrometer (ICP-MS; Varian, Australia) was utilized [22]. Whole blood (5 ml) was collected by a trained technician from 28 exposed and 27 control subjects using sterilized non-heparinized syringes. The blood was homogenized for 10 min by mechanical shaking. Aliquots (0.5 ml) were diluted 1:9 with a solution of 4.5 ml deionized water containing ammonia (0.07 M), Triton-X 100 (500 mg/l), and Na_2-EDTA (500 mg/l). The sample digests were filtered with Wattman paper several times to get a clear solution. The diluted digests were measured directly by ICP-MS and concentrations of nickel and chromium were quantified as micrograms per liter (μg/l).

2.3. Semen analysis

The participants were given written and oral instructions on how to avoid contamination of semen samples. The semen samples were collected into acid-washed glass test tubes. Weekly semen samples were obtained from each participant for a period of 2 weeks. Every sample was obtained through masturbation after a 3-day period of sexual abstinence. All procedures and interpretations used were in accordance with established World Health Organization [23] criteria. The samples were put directly into clean glass containers that were warmed to 37 °C in a dry-heat incubator for up to 30 min. After gentle mixing, the specimen was assessed for liquefaction, volume, and pH. Aliquots of each specimen also were evaluated for viscosity, sperm agglutination, nonspecific aggregation, sperm count, percentage of spermatozoa with motility of grades 1–3 and immotile sperm, and concentration of white blood cells.

Semen volumes were measured with graduated test tubes. The semen viscosity was estimated by introducing a glass rod into the sample and observing the length of the thread formed on removal of the rod. The thread does not exceed 2 cm in normal samples. According to their consistency, the semen samples were considered to have normal or abnormal viscosity [23].

Aliquots of 10 μl semen (five aliquots were analyzed from each sample) were placed on a clean glass slide with a micropipette and covered with a 22 mm × 22 mm glass coverslip. The freshly made wet preparation was left to stabilize for approximately 1 min. Sperm agglutination and nonspecific aggregation were evaluated. Agglutination of spermatozoa was considered to have occurred when motile spermatozoa stuck to each other, either head to head, mid-piece to mid-piece, tail-to-tail, or in a mixed fashion. The adherence either of immotile spermatozoa to each other, or of motile spermatozoa to other cells or debris was considered to be nonspecific sperm aggregation rather than agglutination. Sperm agglutination and nonspecific sperm aggregation were estimated in 10 randomly selected fields per 10-μl drop in the five drops analyzed in each sample, under a light microscope (125× magnification), and the mean percentages were recorded.

Sperm motility was estimated at room temperature within 1 h after ejaculation by counting both motile and immotile spermatozoa in 10 separate and randomly selected fields. At least 100 spermatozoa were assessed per 10-μl drop in the five drops analyzed in each sample under a light microscope (125× magnification), and the proportion of sperm that exhibited flagella activity was noted. Spermatozoa that crossed the microscopic field with very active forward progression and moderate forward progression were recorded, along with those that showed "in situ" motility and no motility. The results were expressed as grade 3, grade 2, grade 1, and grade 0, respectively [23].

Aliquots of the samples were diluted 1:20 using 0.1 M of phosphate-buffered 3% formaldehyde solution, and the sperm concentration was analyzed manually on both sides of a Neubauer hemacytometer. Data were expressed as millions of spermatozoa per milliliter.

Sperm morphology was evaluated on air-dried smears stained with Papanicolaou's stain modified for spermatozoa and examined under a light microscope (1000× magnification). At least 300 sperm per sample were categorized as normal or abnormal according to the presence of head, mid-piece, and tail defects [23].

Sperm viability was assessed after supravital staining with eosin Y-nigrosin (Merck AG, Darmstadt, Germany). One hundred spermatozoa per drop were counted in the five drops analyzed in each sample, and the dead sperm (stained) were differentiated from the live sperm (unstained). The results were expressed as the percentage of live sperm.

A normal semen profile was defined as follows: $>20 \times 10^6$ spermatozoa per milliliter or $>40 \times 10^6$ spermatozoa per ejaculate; ≥50% progressive motility (grades 2 and 3), and ≥30% sperm with normal forms.

Samples were randomized and coded to blind the scorer. The scoring was carried out by one person, to avoid inter-scorer variability.

2.4. Statistical analysis

Log transformation was used for all semen parameters to improve normality of the data. Statistical analyses were performed using the χ^2-test, the Mann-Whitney *U*-test, and Spearman's correlation analyses. The difference in semen parameters between male welders and control men was statistically analyzed by the nonparametric Mann-Whitney test.

3. Results

The mean age of the male welders was 32.3 ± 4.4 years (range 21–41 years) and of controls was 32.2 ± 4.7 years (range 23–40 years). The mean (±S.D.) duration of exposure to welding fumes containing nickel and chromium was 11.2 ± 4.5 years with a minimum of 2 years and a maximum of 21 years.

3.1. Influence of social habits on infertility

Forty-five (40.7%) of the men were smokers and 32 (24.1%) were alcohol consumers. Smoking did not show a significant effect on semen parameters in controls or in welders.

3.2. Semen characteristics

Semen parameters are given in Table 1. No significant difference was found in the volume of ejaculated semen from male welders and control group. Men exposed to

Table 1
Semen characteristics of the control group and welders (mean ± S.D.)

Semen parameter	Control group ($n = 57$)	Welders ($n = 57$)
Ejaculate volume (ml)	2.5 ± 0.5	2.4 ± 0.5
Sperm count ($\times 10^6$/ml)	62.8 ± 43.7	14.5 ± 24.0***
Sperm motility		
Rapid linear progressive motility (%)	63.5 ± 5.3	32.2 ± 15.3***
Slow linear progressive motility (%)	12.5 ± 1.9	21.7 ± 11.5***
Nonprogressive motility (%)	11.6 ± 2.7	15.1 ± 8.9***
Immotile (%)	12.4 ± 7.0	31.0 ± 16.6***
Normal morphology (%)	69.0 ± 8.0	37.0 ± 14.3***
Head defects (%)	16.4 ± 5.6	38.3 ± 9.7***
Mid-piece defects (%)	9.8 ± 3.8	19.5 ± 9.2***
Tail defects (%)	4.8 ± 0.8	5.2 ± 4.9
Sperm vitality (%)	80.4 ± 6.8	67.6 ± 22.8***
Nonspecific aggregation (%)	14.0 ± 12.0	49.0 ± 22.0***

*** $P < 0.001$, Mann-Whitney *U*-test.

welding fumes had a significantly lower sperm count, rapid linear progressive motility, and slow progressive motility when compared to control men. There was a significantly lower percentage of normal sperm, and a significantly higher percentage of head defects and mid-piece defects in male welders when compared to those in the control group.

Sperm vitality was decreased in men exposed to welding fumes compared to those with control men. There was a significantly higher percentage of sperm with aggregation in male welders when compared to the control group (Table 1).

3.3. Correlation between nickel and chromium and sperm parameters

To evaluate the possibility of a dose-dependency, the relationship between individual heavy metal blood concentration and the semen parameters (sperm count, rapid linear progressivity, slow linear progressivity, nonprogressive motility, immotility, head, mid-piece, tail defects, sperm vitality, and sperm aggregation) based on years of exposure and blood levels were statistically analyzed by simple regression analysis in control men and male welders (Table 2). Blood levels were measured in 28 male welders and 27 control men. Therefore, semen values only for these particular individuals were included in the regression analysis. The levels of nickel and chromium in blood of men exposed to welding fumes were significantly higher than those of the control group (Table 2).

3.4. Nickel and sperm parameters

There was a significant positive correlation between the percentage of tail defects and blood nickel concentration in male welders. There was a significantly positive correlation between the years of exposure to welding fumes and blood nickel concentrations ($r = 0.490$, $P = 0.008$). However, there was no significant correlation between blood nickel and the remaining sperm parameters in men exposed to welding fumes or in the control group (Table 2).

3.5. Chromium and sperm parameters

There was a significant positive correlation between the percentage of tail defects and blood chromium concentration in male welders. Sperm count showed negative significant correlation with chromium concentration and rapid linear progressive motility also showed similar correlation.

Sperm vitality decreased with increasing chromium concentration. There was a significant positive correlation between the years of exposure to welding fumes and blood chromium concentrations ($r = 0.516$, $P = 0.005$). There was however no significant correlation between blood chromium and the remaining sperm parameters in welders or control men (Table 2).

4. Discussion

It has been estimated that in industrialized nations about 1% of workers is engaged in the welding of metals. The health effects of welding are therefore of interest, especially the welding of stainless steel where the fumes and dusts generated contain hexavalent chromium and nickel. Several chromate and nickel compounds have been shown to be carcinogenic in animals and humans [24]. Several studies have indicated a worldwide decreasing trend in average sperm counts and sperm quality [25], raising the possibility of a causative role for environmental exposures such as heavy metals.

The results of the current investigation indicated a significant reduction in semen quality of the male welders occupationally exposed to nickel and chromium. These welders

Table 2
Simple linear coefficient between blood nickel and chromium and semen parameters in the control group and in welders (mean blood concentration ± S.D. in parentheses)

Semen parameter	Nickel				Chromium			
	Control group (16.7 ± 5.8 μg/l), $n = 27$		Welders (123.3 ± 35.2 μg/l), $n = 28$		Control group (17.4 ± 8.9 μg/l), $n = 27$		Welders (131.0 ± 52.6 μg/l), $n = 28$	
	r	P	r	P	r	P	r	P
Sperm count	−0.254	0.202	−0.352	0.067	−0.093	0.646	−0.424	0.025*
Rapid linear progressive motility	−0.063	0.757	−0.381	0.045*	−0.035	0.864	−0.482	0.009*
Slow/nonlinear progressive motility	0.150	0.455	0.386	0.042*	0.236	0.235	0.291	0.133
Nonprogressive motility	0.201	0.314	0.141	0.474	0.088	0.662	0.360	0.060
Immotility	0.115	0.569	0.007	0.971	0.204	0.569	0.049	0.805
Normal morphology	0.178	0.373	−0.032	0.872	0.019	0.926	−0.078	0.693
Head defects	−0.101	0.615	−0.145	0.462	0.050	0.804	−0.037	0.853
Mid-piece defects	−0.082	0.684	0.067	0.734	0.029	0.886	−0.011	0.956
Tail defects	0.422	0.028*	0.485	0.036*	0.643	0.001*	0.485	0.009*
Vitality	−0.129	0.521	−0.420	0.026*	−0.107	0.597	−0.507	0.006*

* $P < 0.05$; r: simple linear coefficient.

showed a significant decrease in sperm count when compared to control men. In addition, the men exposed to welding fumes revealed a decrease in sperm motility. Only limited studies have addressed nickel and chromium-induced reproductive dysfunction. In an investigation of 61 welders, semen analysis showed that more than 50% of these men had a sperm count of less than 4×10^6/ml [26]. A study showed reduced sperm count and motility in workers of a chromium-electroplating factory located in China [27]. Poor semen quality and reduced fertility was observed in a Danish study of welders [28]. A recent study has shown that paternal exposure to chromium (III) is accompanied by an increased incidence of aging-associated neoplastic and non-neoplastic abnormalities [29]. It has been reported that stainless steel welders had an increased risk of reduced sperm quality [30]. This increased risk is attributed to the fumes generated by the welding of stainless steel. Welding fumes contain a number of heavy metals, of which chromium is found in high concentrations. The above reports are in agreement with our results.

One of the main findings of our study was that the men exposed to nickel and chromium had a large number of morphologically abnormal spermatozoa in their ejaculates. A previous study reported a lower percent of progressive sperm and a significantly higher percent of coiled tail defects in welders compared to controls [31]. The significant reduction in sperm motility and the increase in tail defects in our results are consistent with results of investigations that have focused on reproductive function in welders [30,32]. Mortensen's [30] multi-center case-control study with 55 welders found an increased risk of seminal abnormalities associated with welding exposure. Results from investigations conducted in Denmark also support the association of welding with decrements in semen parameters, including sperm motility and morphology [32]. However, another Danish study showed no difference in semen quality between welders and non-welders [33]. Similarly, no evidence of deterioration in conventional measures of semen quality was found in a nationwide sample of Danish welders [34]. Further, an investigation of patients from two infertility clinics of The Netherlands revealed that there was no association between metal exposure and abnormal semen parameters [35].

The results of the current study suggest that the presence of nickel and chromium in the blood of the male welders are associated with their reduced semen quality. In a previous study, the concentration of chromium in seminal fluid was significantly elevated among welders compared with non-welders [30]. Several reports on the reproductive effects of lead have suggested an association between lead concentrations in blood and semen with infertility. From among them, Alexander et al. [13] reported a significant association of blood lead with decreased fertility. In contrast, a study found no association among lead exposed workers with blood lead ranging from $<40\ \mu g/dl$ to $>60\ \mu g/dl$ [36].

In our study, sperm count showed a negative correlation with blood chromium concentration in men exposed to welding fumes. A significant negative correlation was observed between progressively motile sperm and nickel and chromium blood levels in male welders. Slow/nonlinear progressive motility was positively correlated with nickel content in these welders. Tail defects also were correlated with nickel and chromium blood levels in the control group and in the male welders. Viable sperm decreased significantly with the increase in nickel and chromium in welders. Significant reductions in human semen quality (decreased sperm density, total, motile, and viable sperm counts, the percentage and count of progressively motile sperm, and increased abnormal sperm head morphology) with moderate exposures to lead in healthy workers have been reported [12]. However, another study did not demonstrate a correlation between semen lead concentrations and sperm morphology [37].

In the current study, smoking did not show an effect on semen parameters in welders or in control men. Alcohol consumption also had no effect on semen parameters. Similarly, another study also demonstrated no significant difference in semen parameters between smokers and non-smokers and no significant influence of alcohol intake on semen quality in men exposed to lead [12].

From our findings it can be concluded that welding is associated with abnormal semen parameters and might affect the reproductive success of welders. Exposed workers with normal semen parameters may be fertile; it is not possible from our data to evaluate possible effects of exposure on fertility, which was not directly assessed. Additional studies on the association between nickel and chromium and male fertility will be necessary.

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