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Semen Quality And Sperm Morphology Among Occupational Solvent Exposed Workers.

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Abstract

Objective: To study the effect of organic solvents on male semen quality among the shoe manufacturing industrial workers.

Design: Approached recognized clinicians, Doctors for authentication of the clinical data. Pub Med/Medline, Standard research articles were searched for systematic review.

Setting: The wok was set up in collaboration with the infertility centers and with the ESI hospital.

Patient(s): None

Intervention(s): None

Main Outcome Measure(s): 40 male workers with a mean age of 34.13 ± (6.72) years and a mean organic solvent exposure of 9.70 ± 4.32 were recruited in this study. The semen samples were obtained by masturbation and processed for gross conventional semen analysis.

Result(s): The result of this study indicates a significant higher number of morphologically abnormal sperms and the percentage of motility among the solvent exposed workers with respect to the control. However there was no significant alteration noticed in the semen volume, liquefaction, pH and sperm viability between the exposed and unexposed workers.

Conclusion(s): The results suggest that the organic solvents could affect the semen quality of the workers in terms of sperm morphology which might be considered as one of the reason for abnormal pregnancies among the wives of the workers. Due to various limitations of the study there is a need to have a complete investigation to draw an authentic conclusion.

Keywords: organic solvents, sperm morphology, occupational exposure

Introduction

Occupational exposure to toxic agents is one of the most prevalent occupational and environmental health problems in the world today. Epidemiological studies have revealed that the occupational exposure to toxic agent leads to reproductive toxicity such as poor semen quality [1] and male mediated adverse pregnancy outcomes such as spontaneous abortions, congenital malformations and premature births [2,3].

Organic solvent is an important occupational health problem due to its wide spread usage in industries like shoe making, spray painting and paint manufacturing. There are certain operations where organic solvent exposure is significant and constitute a health hazard to the workers in shoe manufacturers. These operations are mainly waxing, gluing and polishing [4,5]. Exposure to organic solvents in shoe factories has been reported to increase the risk for acute and chronic health problems [6]. Higher incidences of cancer are reported in subjects exposed to solvents characterized by its reactive metabolites which are presumed to induce oxidative damage on vital organs and to the hematopoietic system [7].

Several studies on human reproduction against solvent exposure reveals that there is an association between paternal occupational exposure to solvents and abnormal pregnancy outcome and can cause testicular damage and also impairs the spermatogenesis [8,9] spontaneous abortions [10], low birth weight and defects [2]. Although the literature related to the

effect of specific substances on semen quality, the relationship between solvent exposure and male infertility is less well documented. Only few studies have explored the information on the effect of solvents on sperm quality among the exposed workers to perchloroethylene [11], Trichloroethylene [12], Styrene [13] and Ethylene glycolethers [14]. Since the semen parameters includes the volume, sperm concentration, motility and morphology are considered as valuable and widely accepted indicators of exposure to occupational and environmental factors that affects the male fertility. Hence the present study was performed to see the possible effects of organic solvents on semen quality of shoe making workers from a leather shoe factory situated at IDA Nacharam.

Materials and Methods

Air Sampling and Analysis: Air samples were collected from the industry to detect the air level concentrations of organic solvents by using KIMOTO air sampler for three consecutive days. The concentration of the organic solvents of toluene, gasoline, acetone and benzene were measured by vapour phase chromatography (GD-14A, Shimadzu, Japan). The mean concentrations of toluene, gasoline, acetone and benzene in work shift air borne was 230 ± 60, 300 ± 120, 525 ± 230 and 103.34 ± 45 mg/m^3 respectively.

Study Population: The subjects were selected from a shoe manufacturing industry situated at IDA Nacharam of Ranga Reddy district of Telangana in India. 40 male workers from the industry were recruited with a mean exposure period of 9.70 ± 4.32 and the mean age group of 34.13 ± 6.72. In addition, 40 aged and occupationally matched non exposed controls were selected for comparison. The workers participated in the study only once. All the subjects were interviewed for personal data, duration of employment, data on previous medical and occupational history, life style factors through a standard WHO questionnaire. In particular the male workers were asked about any illness (or) condition that would impair their ability to become a father such as parotitis, varicocoele. Only 30 out of 40 exposed shoe making workers were volunteered to donate the semen sample and 40 controls volunteered for the sampling. This study was approved by the local ethics committee. Informed written consent was obtained from each subject. An institutional review board approval was obtained prior to starting of the work.

Sample collection and analysis: Each worker was asked to provide the semen sample by masturbation for the assessment of the semen quality parameters. The workers had been asked to abstain for a period of 3days before the sample collection. Samples were collected into a sterile wide mouth and metal free plastic container. The samples were shifted to the laboratory immediately after collection and was incubated at 37 °C until liquefaction completed. The collected semen volume was measured to the nearest 0.1ml and routine semen analysis was performed within 1hour after liquefaction according to the standard method [15]. The pH of the sample was determined within 1hour of ejaculation by placing a drop of semen on the pH paper strip and the colour was compared with the colour of the strip ranging the pH scale 6-9 .The liquefaction time was determined in minutes. Sperm motility was assayed by placing a drop of semen on prechilled cleaned micro slide within 30-60 minutes after the collection of the sample and observed under the light microscope. The viability of the sperms was determined by using 1% trypan blue solution. Sperm concentration was determined by using haemocytometer. For this purpose 20 microlitre of semen sample were taken and diluted with sperm diluents. The sperm count were made in the five central squares. The slides for the assessment of sperm morphology was prepared on the clean microslide and stained with papanecolus staining procedure. Minimum of 50-200 sperms from each subject were analyzed for sperm morphology.

Statistical analysis: Statistical analysis was carried out by using student–t test in order to obtain the significance between the two groups and also to find out the correlation between the various parameters.

Results

In the present study deterioration of sperm morphology was more evident among the occupational workers. However there is no distinction found between the control and exposed group in other semen parameters. The overall results on mean pH value, liquefaction time, semen volume did not show any statistical significant difference between the two groups. The appearance of the semen was normal in all the subjects. The results indicate that the sperm concentration was above 20million/ml in the subjects studied and the sperm concentration among the workers are 85.34 ± 73.68million/ml as against 82.16 ± 43.58 in control. The sperm count among the exposed workers was significantly less (12.00 ± 3.40) as compared to the control group (65.00 ± 20.90).

A significant decrease in percentage of sperm motility parameters (included rapid and progressive) were observed among the exposed workers compared to the controls. The solvent exposed subjects showed the motility below 50% with the range of the motility was 10.25 to 50. The mean percentage of the sperms with motility rapid/progressive (16.79 ± 3.96), sluggish (10.25 ± 5.94) and immotile (50.0 ± 7.23) were significantly ($P<0.05$) higher than the control group (80.23 ± 23.59, 16.16 ±8.81 and 2.9 ± 0.65 of rapid, sluggish and immotile respectively).

The sperm morphology reveals that a gross deterioration in the sperm structure among the exposed group as compared to the control subjects. The mean morphologically normal sperms was statistically ($P<0.05$) significantly low in shoe factory workers when compared to the respective controls. 86.20 ±2.24 percentage of abnormal sperms were reported among the

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workers as compared to 18.80±1.67 in control groups. The mean percentage of the sperms with morphologically abnormalities such as thick coiled tail (38.2 ± 7.60 vs 3.45 ± 1.44), amorphous head (20.60 ± 4.18 vs 5.20±0.79), tapered head (23.0 ± 5.82 vs 6.18±0.06), double head (5.14 ± 1.12 vs 1.18 ± 0.39), dilated middle piece (3.81 ±0.25 vs 1.12±0.25) and short thick tail (3.0 ± 1.04 vs 1.40 ± 0.05) were significantly higher in shoe factory workers than in the control group ($P<0.05$). The average total sperm count in shoe factory workers was 12 million which was statistically significantly lower than the control group of 65 million/ml.

Discussion

Occupational exposures to toxicants may impair male reproductive health and cause infertility in humans [16]. Several studies have suggested that human semen quality is declining. However the relationship between chemical exposure and male infertility is more contradictory. Organic solvents are the most important occupational reproductive hazards. Occupational exposures to organic solvents are wide spread and the risk of infertility is not clear. Exposure to solvents brings an alteration in human seminal quality [17], [18]. The evidences in the scientific literature on the outcome of organic solvent exposure in male fertility is inconsistent. A change in sperm morphology among dry cleaning workers exposed to perchloroethylene [11] and a non significant decrease in fecundability among the workers exposed to organic solvents [9] were reported.

Our findings are in accordance with the findings of several earlier studies which have shown a weak association [9,19] and others have been negative [20,21]. Our study further corroborates with an earlier studies on solvent exposed population [22,23]. This study was further supported by the observations made among chromium workers [24,25] welding workers [26] and fenvalerate exposed workers [27] against male infertility.

The effect of solvents on the sperm quality have also been inconsistent and inconclusive. The deterioration of sperm quality among the workers observed during the study may be as a resultant of increased risk of Oligospermia, Azoospermia. Similar findings were reported among shipyard painters exposed to ethylene glycol ether [14] and 2- ethoxy ethanol [28]. Further a lower sperm density was reported among the workers exposed to organic solvents which was supported by the observations among the trichloroethylene exposed workers [12]. The functional competence of sperm and production of required number of sperms are essential to ensure the fertilization. The progressive and sustained motility of the sperm is an important function of sperm and the evaluation of sperm motility will provide an useful information for evaluating the chemical effects on male fertility [29]. In the present study the routine semen analysis and sperm motility were significantly decreased with a significant difference in sperm count which was supported by the observations on solvent exposure workers [22]. Some of the reproductive toxins may affect the motility of the sperm, without altering the other seminal parameters [30].

The morphology of the spermatozoa is the end result of the spermatogenesis [31]. The examination of the sperm morphology is an important parameter to evaluate the sperm function. The observed changes in sperm morphology in the study was in agreement with that of the observations among the styrene and acetone exposed workers [32] and plastic workers exposed to styrene [33]. The noticed change may be as a result in the disruption of testicular physiology leading to reproductive impairment. In this present study we did not find any gross alteration in sperm parameters in relation to organic solvent exposure, however a significant association between deterioration in sperm morphology and percentage of motility was observed. At this moment it is difficult to draw a conclusion about the mechanism of the observed changes in sperm morphology however the sperm DNA damage may be one of the possible mechanism for the observed changes in our study and is known that DNA damage may result in cell death and may be due to apoptosis which has been found to occur in testis during spermatogenesis [34]. The poor semen quality reported in this study may be the result of the gonadal endocrine axis disruption [35] or the spermatogenesis process [36], [37].

In conclusion organic solvents have an adverse effect on male workers semen quality. Due to various limitations of this study (the size of the sample) the results warrant further investigations.

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Conflict of Interest

We the authors of this research work is here by solemnly declare that this work is not having any conflict and if any potential conflicts in the interest in favour of this, we will take the responsibility.

Table 1: Characteristics of the study groups (Mean ± SD)

Group	N	Age(yrs)	Marriage(yrs)	Duration of employment (yrs)
Industrial workers	60	34.13 ± 6.72	8.11 ± 3.92	9.70 ± 4.32
Control	60	30.65 ± 6.23	10.00 ± 6.11	-

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Table 2: Concentration of air borne organic solvent concentration in the ambient air (mg/m3)

Solvent	Air borne concentration (mg/m3)
Toluene	230 ± 60
Gasoline	300 ± 120
Acetone	525 ± 230
Benzene	103.34 ± 45

Table 3: Comparison of semen parameter among the study group.

Group	N	Volume (ml)	pH	Liquefaction	Sperm density (mean x106/ml)	Sperm count (x106/ ejaculation)	Motility			Sperm morphology		t-test
							A	b	C	Normal mean±SD	Abnormal mean±SD	
Industrial workers	30	2.54 ± 1.47	7.28 ± 0.28	32.62±11.76	85.34±73.68	12.00 ±3.40*	16.79 ± 3.96*	10.25 ± 5.94*	50.0 ± 7.23	14.87±2.5*	86.2 ± 2.24*	<0.05
Control	40	2.90 ± 0.95	7.35 ± 0.29	34.29±16.97	82.16±43.58	65.00 ± 20.90	80.23 ± 23.59	16.16 ± 8.81	2.9 ± 0.65	82.05 ± 13.4	18.8 ± 1.67	

Motility - a- Rapid/progressive; b- sluggish; c - Immotile.

the experimental results are found

1. there is no statistical difference between the control and exposed group in volume, liquefaction pH, sperm viability.
2. Effect of solvent on sperm morphology, motility was siginificant at P< 0.05.

Table 4: classification of abnormal sperm morphology.

Type of Abnormality	Control	Exposed	t-test
Thick coiled tail (%)	3.45 ± 1.44	38.2 ± 7.60*	<0.05
Amorphous	5.2 ± 0.79	20.6 ± 4.18*	
Tapered head	6.18 ± 0.06	23.0 ± 5.82*	
Pinpoint head	1.18 ± 0.39	5.14 ± 1.12*	
Dialated middle piece	1.12 ± 0.25	3.81 ± 0.25*	
Short thick tail	1.4 ± 0.05	3.0 ± 1.04*	

Values are expressed as Mean ± SD

P<0.05*

various types of sperm head and middle piece and tail abnormalities were

significantly higher (<0.05) among the workers as against control.

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