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The evaluation of the effects of occupational arsenic exposure on man reproductive hormones

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Abstract

The aim of this study is to determine the relationship between arsenic levels and reproductive hormones of workers with occupational arsenic exposure. Forty arsenic exposed workers, who applied to Ankara Occupational Disease Hospital, Occupational Health Outpatient Clinic between 2013-2017 with no complaints about infertility and erectile dysfunction (ED), were included in this study. Arsenic exposed individuals in the study group were working in the recycling and pest control companies. A healthy group, who consists of 57 office workers with no heavy metal exposure at workplace, was selected as the control group. Workers who have chronic disease, prescripted or herbal medicines were not included in this study. Whole group was composed of 97 male subjects, with 40 arsenic exposed workers and 57 control subjects. In the study group, urine arsenic levels (UAL) was significantly higher than the control group (57.98±26, 70 µg/L and 12.84±6.82 µg/L respectively) (p=0.000). Serum follicle stimulated hormone (FSH) and luteinizing hormone (LH) levels were found to be higher in the study group (FSH: 4.52±2.08/3.58±2.10, LH: 4.66±1.92/ 4.20±1.92). Total testosterone (TT), free testosterone (FT), and prolactin levels were significantly lower than in the control group (TT: 5.63±2.01/6.80±2.43, FT: 10.31±2.87/16,74±4,32, Prolactin: 12.61±7.03/15.55±6.93). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), thyroid stimulating hormone (TSH), uric acid (UA), creatinine, triiodothyronine (T3) and thyroxine (T4) levels were similar in both groups. In the complete blood count (CBC) parameters, the HGB and HTC values were found lower in the study group. This is a pilot study that shows the toxic effects of arsenic exposure on male reproductive hormones.

Keywords: Testosterone, arsenic, occupational exposure, Prolactin, follicle stimulated hormone (FSH), luteinizing hormone (LH)

Introduction

Arsenic (As) compounds are widely used in the production of paint, glass, ceramics, semiconductors and agrochemicals. It is a toxic metalloid which is a natural part of earth crust [1-3]. Most prevalent arsenic is inorganic types found mainly is waters. Organic form of it are found in natural gas and petroleum source. As changes its chemical forms and oxidation level easily by the effect of microbiologic activity, redox potential, water pH and the presence of ions [4].

Leading routes of As exposure are production of ceramics, mining process, herbicides and insecticides [5]. Arsenic enters the human body via ingestion or inhalation or absorption by skin. Ingested arsenic is easily absorbed through gastrointestinal tract. Inhaled arsenic is also well absorbed by lungs and enters bloodstream. First target of arsenic compounds is erythrocyte in body after systemic absorption [6]. Chronic arsenic exposure leads to metabolic and structural changes in hepatocyte and mitochondria in the liver as well as apoptosis, oxidative damage and lipid peroxidation [7-9]. It has been proven that As is clinically relevant bladder, lung, liver and skin cancers and it is defined as Group 1 carcinogen by the IARC (The International Agency for Research on Cancer) [10].

Adverse effects of As on circulation, respiration, digestion, hematology, musculoskeletal system, neurological and reproductive system have been proven by many scientific researches [7,11-13]. Effects of As exposure on reproductive system is mostly studied by animal models. Mehranjani and Hemadi [14] reported a statistically significant reduction in FSH and LH concentrations in rats treated with sodium arsenide at 8 mg kg BW-1 daily dose for eight weeks. Ali et al. found that FSH and LH levels increase and testosterone levels decrease in As exposed mices [15]. In another study with

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wistar rats, the plasma levels of FSH, LH and testosterone were found to be decreased with intraperitonally injected sodium arsenite [16]. However, Chiou et al. observed no FSH level change in arsenic trioxide injected mice although testosterone and LH levels decrease [17]. In the same study epididymal sperm number, motility and viability were found significantly decreased.

In several animal studies, it has been found that arsenic causes a significant decrease in testicular weight, epithelial degeneration in seminiferous tubules, and loss of both Leydig and epithelial epididymal cells [1,18,19]. However, there is a limited number of studies about the effects of As exposure, both occupational or environmental, on the male reproductive system in humans [2,20-22].

In this study total testosterone (TT), free testosterone (FT), follicle-stimulated hormone (FSH), luteinizing hormone (LH) and prolactin were measured and compared with urine arsenic levels of As exposed workers in order to show effect of As on male reproductive system. This is a case-control study that investigates the effects of occupational arsenic exposure on male reproductive system.

Material and Methods

Study Group

Forty arsenic exposed volunteer workers with a mean age of 39.57, who applied to the Ankara Occupational Disease Hospital, were selected as study group, 31 people were recycling workers and nine were pest control workers. Healthy office workers, with a mean age of 33.07 with no exposure to arsenic or any toxic material, were chosen as control group. Both groups consist of non-smoker male subjects with over 18 years old. This study was approved by Ankara Keçiören Training and Research Hospital Research Ethics Committee of Health Sciences University (Decision No: 2012-KAEK-15/1619).

Measurements

All blood samples were taken to Ethylenediamine Tetra Acetic Acid (EDTA) tubes. Haematological analyses were carried by Cell-Dyn Emerald Hematological Analyzer. Arsenic levels were measured by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) Agilent 7700 instrument. The levels of FSH, LH, PRL and testosterone were determined by Abbott Architect i2000 Immunoassay instrument. Serum AST, ALT were analyzed by spectrophotometric methods on Roche E170 Modular System (Roche Diagnostics, Mannheim, Germany).

Statistical Analysis

The data were statistically analyzed by SPSS 22.0 software. The normal distribution convenience of the data was determined by the Kolmogorov-Smirnow test. In the study, the statistical significance level was accepted as 0.05. The difference between the averages of the two independent variables and the correlation between two variables were evaluated by the t-test and Pearson Correlation analysis, respectively.

Results

The laboratory findings of the participants were presented in Table 1. There were 40 As exposed workers in study group and 57 workers in control group. The mean UAL of exposed group was significantly higher than of control group, (57.98±26.70 µg/L and 12.84±6.82 µg/L respectively) (p=0.000).

FSH and LH levels were found to be higher (FSH: 4.52 ± 2.08 / 3.58 ± 2.10, LH: 4.66 ± 1.92 / 4.20 ± 1.92); TT, FT and prolactin levels were lower (TT: 5.63 ± 2.01 / 6.80 ± 2.43, FT: ± 2.87 / 16.74 ± 4.32, Prolactin: 12.61 ± 7.03 / 15.55 ± 6.93) in the study group.

When both groups are examined; there were no significant differences in ALT, AST, TSH, uric acid, creatinine, T3 and T4 levels.

In our study, it was found that the levels of Hgb and Htc decreased in the exposed group that is similar to the results of some other researches [3,23,24].

Table 1. Laboratory findings of the groups

Parameters	Arsenic exposed group (n=40)	Control group (n=57)	p value*
Urinary arsenic (µg/L)	57.98±26,70	12.84±5.79	0.000
Total testosterone (ng/dl)	5.63±2.01	6.80±2.43	0,014
Free testosterone (pg/ml)	10.31±2.87	16,74±4,32	0.000
FSH (mIU/ml)	4.52±2.08	3.58±2.10	0.033
LH (mIU/ml)	4.66±1.92	4.20±1.92	0.250
Prolactin(U/L)	12.61±7.03	15.55±6.93	0.044
TSH (µIU/ml)	1.43±0.75	1.38±0.69	0.767
T3(pg/ml)	3.08±0.44	3.11±0.39	0.723
T4(ng/dl)	1.06±0.15	1.03±0.11	0.301
ALT (U/L)	21.97±10.65	20.80±12.59	0.634
AST (U/L)	19.22±4.48	18±4.89	0.212
Uric asit (mg/dl)	5.47±1.12	5.16±1.08	0.180
Creatinine (mg/dl)	0.82±0.15	0.81±0.10	0.851
HGB (g/dl)	15.40±1.07	15.92±1.22	0..034
HCT %	45.73±2.97	47.29±3.40	0.021
WBC(kµ/L)	7.27±2.10	7.63±1.70	0.350
Age (years)	39.57±5.79	33.07±8.13	0.000

*; p<0.05 is statistically significant, values are presented as “mean ± SD”

The Pearson Correlation coefficients (r) of continuous variables are presented in Table 2. There was a negative correlation between UAL and free testosterone (r = -0.263, p <0.01).

Regression analysis, in which FT is dependent variable, age and UAL is independent variable, showed that UAL and age are responsible 32.4% of the change in FT. The results of the regression analysis and obtained formula are presented in Table 3.

Table 2. Pearson correlation of continuous variables

		Age	UAL	FT	TT	ALT	AST	TSH	Cre	T3	T4	UA	HGB	HCT	FSH	LH	PRL
Age	**R**	1	.245*	-.416**	-.036	.005	.069	.111	.063	-.189	.012	-.063	-.137	-.165	.289**	.239*	-.220*
UAL	**R**		1	-.263**	-.023	.004	-.014	.178	-.071	-.084	-.052	-.083	-.003	-.008	.101	.051	-.075
FT	**R**			1	.383**	-.050	-.140	.023	-.058	.116	-.001	-.139	.105	.126	-.161	-.162	.278**
TT	**R**				1	-.112	-.130	.087	-.091	-.070	-.011	-.230*	.090	.115	-.143	.074	.035
ALT	**R**					1	.783**	-.049	.059	.255*	-.018	.150	.118	.163	.013	-.068	-.199
AST	**R**						1	-.054	.127	.202*	-.008	.139	.039	.099	.013	-.017	-.141
TSH	**R**							1	.217*	.115	.105	-.128	-.052	-.048	.258*	.159	-.037
Cre	**R**								1	.133	.259*	.228*	.031	.091	.269**	.187	.005
T3	**R**									1	.223*	.110	.314**	.369**	-.073	-.084	-.120
T4	**R**										1	.008	-.075	-.074	.220*	.083	-.181
UA	**R**											1	.004	-.067	-.015	-.038	.026
HGB	**R**												1	.896**	-.100	-.044	-.034
HCT	**R**													1	-.175	-.128	-.086
FSH	**R**														1	.516**	-.074
LH	**R**															1	.096
PRL	**R**																1

Table 3. Regression analysis results

Dependent Variable	Independent Variable	F	Beta	t	R2
FT	Age	22.499*	-0.287	-3.219*	0.324
	UAL		-0.409	-4.581*	
	Formula	FT= 22.729-0.179.age-0.071.UAL			

*p<0.001

Discussion

Toxic effects of As on human tissues and body systems are well documented by both human and animal studies. However, studies about its effects on reproductive system are very limited. Manna et al. found degeneration of seminiferous tubules and defoliation of spermatocytes in orally sodium arsenite given mouse [25]. Decrease in sperm count and increase in abnormal sperm in the epididymis were found after gallium arsenite intratracheal installation in rats [26]. Sperm abnormalities, decrease in serum testosterone and prostate cancer were reported in occupationally arsenic exposed men [27,28].

Ali et al. observed significant reduction in sperm count and motility and also declined level of testosterone and LH in As given mice [16]. Sarkar et al. reported reduction in LH, FSH and testosterone level and deterioration in germ cells of testicular tissue due to arsenic toxicity [29]. Depletion of testes weight, LH, FSH, testosterone and semen parameters also observed in As treated rabbit bucks [30]. Momeni and Eskandari found reduced sperm number, motility, viability and morphology in sodium arsenite treated rats [31]. Reduced testes, epididymis, prostate and seminal vesicle were found in sodium arsenite given swiss albino rats [32]. FSH, LH and testosterone levels were found decreased in the same study.

In another study although no significant change in testis weight was observed, reduction in total motility of sperm, rapid moving spermatozoa, moderately moving spermatozoa and slow moving spermatozoa was present in arsenic treated animals [33]. It was shown that if blood As levels higher than 5.8µg/L resulted in decrease in sperm motility [34].

In our study, in the presence of high As levels, testosterone levels decrease with an increase in FSH ($p < 0.05$) and LH ($p = 0.250$) levels. These findings suggest that As causes testicular damage with direct toxic effect and reduces testosterone synthesis, and this status results in compensatory increase in FSH and LH concentrations. Our results also support the animal studies showing formation of sertoli cell damage due to arsenic exposure [1,2,19,20].

In a study conducted by Meeker et al. [35], the LH level was found to be lower when arsenic level was increased in blood. Hsieh et al. found that the increase in arsenic exposure caused decrease in testosterone levels in 177 men. They found significant ED in individuals with FT level <0.23 nmol / L. The mean testosterone and free testosterone levels were found to be lower in patients living in As endemic areas and having arsenic exposure greater than 50 ppb concentration [21]. There were no clinical complaints in our study group, but according to the obtained results, it can be considered that these individuals are vulnerable for the development of clinical outcomes such as ED due to testosterone release damage.

The toxic effect mechanism of As exposure on the endocrine system is not clear. However, the oxidative stress during metal exposures is thought to be one of the important causes of this toxic mechanism [36,37].

Anterior pituitary gland plays an important role in the reproductive system. In animal studies, As has been shown to affect gonadotropin levels by showing hypothalamic pituitary axis or anterior pituitary toxic effects on the hypothalamus. Meeker et al. [38] showed that there was a significant decrease in prolactin levels by arsenic exposure in 219 male volunteers. Similarly, in the study by Jahan et al. prolactin levels were found to be significantly reduced in arsenic exposed mice [39]. Sonia et al. [40] showed that arsenic induced cellular apoptosis by the effect of oxidative stress in

the anterior pituitary gland. In their study on mice, they found significant decrease in prolactin levels with increased arsenic level. Parallel to the results of these studies, we found statistically lower prolactin level in the arsenic exposed group. Our results also support toxic effect of arsenic on the hypothalamic pituitary axis.

In addition to reproductive system effects, It is known that chronic arsenic exposure also causes hepatocyte damage in the liver [8,9]. In our study, although they were not statistically significant, AST and ALT levels were high in arsenic-exposed group, which may be consistent with possible hepatocyte damage of As exposure.

Conclusion

Our results showed that chronic As exposure creates the toxic effects on the male reproductive system by reducing testosterone release. FSH and LH seem to show a compensatory elevation reflecting decreased testosterone release.

Although there is no clinical findings in study group, there could be a certain degree of damage and its effects may become clinically apparent in more severe and prolonged As exposures.

Competing interests
The authors declare that they have no competing interest.

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Ethical approval
This study was approved by Ankara Keçiören Training and Research Hospital Research Ethics Committee of Health Sciences University.

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