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The Effect of Aflatoxins on Male Reproduction

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ABSTRACT

Background: idiopathic male infertility is a cause for increased concern of health caregivers. Semen quality abnormalities are not well understood; environmental factors such as aflatoxins may play a role. **Aim:** the study conducted to determine the effect of Aflatoxins on male production. **Methods and subjects:** semen samples from 108 adult men were examined for aflatoxins. Of whom, 60 males with infertility and 48 fertile males served as controls. Semen was screened for the presence of aflatoxin using solvent extraction by high performance liquid chromatography. Seminal parameters were determined according to the WHO criteria for semen parameters. Statistical analyses were done using t-test and x^2 test. **Results:** The prevalence of aflatoxins in the infertile males was 25% (15) compared to 2.1% (1) in the fertile males (P=0.0007). The majority of infertile men with aflatoxins had a high level of toxicity compared to fertile group (53.3% vs. 0%, P=0.0202, respectively), while low and medium toxicity were similar in infertile and the fertile group, (20% vs. 2.1%, P=0.6274), and (26.7% vs.0%, P=0.1502), respectively. Semen parameters of infertile men were significantly reduced compared to fertile group in volume (2.08 ± 0.84vs. 2.52 ± 0.53,P=0.0021), sperm count(86.4 ± 77.39 vs. 185.37 ± 137.82,P<0.0001), sperm morphology(34.83 ± 12.51 vs. 63.53 ± 6.46,P<0.0001), sperm motility(23.17 ± 11.7 vs. 53.33 ± 7.23, P<0.0001), and viscosity (0.7±.1vs. 1.1±0.01, P=0.0001). **Conclusion:** high percentage of aflatoxins was demonstrated among infertile males with significant reduction of al semen parameters raising greater concern about the relation between idiopathic infertility in male and aflatoxins.

Key words: aflatoxins, male, infertility, semen

1. INTRODUCTION

Globally, human fertility is declining; a situation that cannot be attributed solely to an increase in contraception [1].The decline in semen quality has a significant negative impact not only on male fertility but also on public health. While the exact causes of the decline in semen quality is not yet known, environmental factors have been considered to play an important role [2].

Many environmental agents were postulated to contribute to this decline in male reproductive health [3]. Studies on the effects of environmental agents on semen quality in man have mainly focused on organic toxicants with potential endocrine disrupting activity, but molds may have influence as well. Aflatoxins are secondary metabolites produced by a large number of Aspergillus species, basically *A. flavus*, and *A. parasiticus*, naturally occurring mycotoxins [4]. About 4.5 billion of the world's population is exposed to aflatoxins because they are everywhere [5].

The International Agency for Research on Cancer classified aflatoxins as group 1carcinogen and further proved that chronic aflatoxin exposure causes hepatocellular Carcinoma(6).In human, chronic exposure can lead to potentially lethal hepatitis [7].The damage produced by aflatoxins is due to adduct formation with RNA, DNA and protein. In addition, it also causes free radicals that cause damage to DNA. Aflatoxins B1 has a genotoxic potential in many of test systems [8]. Other aflatoxins are not so extensively investigated; researches have proved that some of them have shown evidence of genotoxicity. [9] All previous studies that were performed on animals proved that aflatoxin could decrease motility of sperm obtained from ejaculation or epididymis [10-12].

Literature on the role of aflatoxin on human reproduction is scant .The current study was conducted to determine the presence of aflatoxins in semen of men with idiopathic infertility and to establish the relationship between aflatoxin and semen parameters.

2. PATIENTS AND METHODS

This study is a case-control hospital-based study that was conducted from December 2011 to December 2012. Sixty patients with idiopathic male infertility (idiopathic oligo and/or asthenozoospermia) were recruited randomly from the infertility clinic at Sohag University Hospital. Exclusion criteria included infertile male with specific genital diseases that may impair reproductive capacity, such as clinical varicocele, genital infection, undescended testis and testicular atrophy. Moreover, we excluded patients with other systemic diseases such as hepatic, renal, endocrine and autoimmune diseases as well. Smokers or

those at risk for exposure to heavy metals were excluded. We included apparently healthy 48 fertile men as controls. The Scientific Research Ethics Committee at Sohag Faculty of Medicine approved the study.

The steps and aim of the research were explained to participants before signing an informed consent. Demographic data was collected through an extensive questionnaire regarding occupation, residence, social status and smoking habits from each participant. Detailed medical history was taken from each participant on special emphasis on sexual history. They were also subjected to thorough general medical and genital examination. All semen samples were collected by masturbation in polypropylene containers after three to 6 days of sexual abstinence. Semen samples were at 37 °C, semen analysis was carried out according to World Health Organization guidelines [13]. The evaluation of the semen included, liquefaction time, pH, viscosity, odor, sperm morphology, presence of pus or epithelial cells, sperm motility, and sperm concentration. Semen findings were categorized as oligozoospermia, a sperm concentration less than 20×10 /ml; asthenozoospermia was defined as fewer than 50% progressive motile sperm; and oligoasthenozoospermia, including both criteria. The samples were extracted from the semen using ethyl acetate (20 ml). The solvent was decanted off and re-extracted by another 10 ml of diethyl ether. The extracts were combined, filtered through filter paper (Whatman No. 1) containing 5 gm anhydrous Na2So4, concentrated in a vacuum , and the dry material was transferred to a dry vial with a small amount of the solvent, which was evaporated to near dryness under a stream of nitrogen. All these samples were examined in the Faculty of Science, Department of Botany laboratories for detecting the presence of aflatoxins and for evaluating the different levels of aflatoxins in each sample. The degree of toxicity was determined using the Brine Shrimp test [14]. A sample size of 53 subjects in each arm of the study was calculated using the OpenEpi-Epidemiological calculator with 80% power and a confidence interval of 95% to detect a difference of 5% at α = 0.05, with 10% of non respondents or incomplete data.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS 15 for Windows) was used for data recording and statistical analyses. The descriptive analyses used included the mean, standard deviation, and frequency distribution. Student t-test was used to compare means of continuous variables, and Chi-square was used to compare the association between categorical variables. The results were considered statistically significant when the P values were <0.05, <0.001, and more than 0.05, respectively.

3. RESULTS

The mean age for the infertile group was 31.5 years compared to 31.0 years in the control group, with no statistical difference (P=0.494). Similarly, there was no difference between the two groups in occupation (P>0.05). The study and the control groups had the same duration of marriage, 6.82 ± 3.67 and 7.15 ± 3.39 years respectively, (P= 0.6321) as presented in table 1.

Variable	**Study group (n: 60)**	**Control group (n: 48)**	P- value
The mean age (years)	31.510 ± 5.299	31.016 ± 5.720	0.494000
Mean duration of marriage (years)	6.82 ± 3.67	7.15 ± 3.39	0.6321
Occupation			
Laborer	43(71.7)	34(70.8)	0.9689
Employee	17(28.3)	14(29.2)	0.9436
Type of infertility			
Primary	38 (63.34%)		
Secondary	22 (36.67%)		
Occupation			

Table 1. Comparison of sociodemographic characteristics between the study subjects the controls. Data are presented as mean±SD, and number (%),p value was set significant at less than <0.05

Semen variable	Infertile patients (n=60)	Control group (n=48)	P-value
Semen volume (ml)			
Range	1 – 4	2–4	
The mean of semen volume	2.08 ± 0.84	2.52 ± 0.53	0.0021
Sperm Concentration (mil/ml)			
Range	2 – 150	20 – 220	
Median	20	65	
Mean ± SD	53.4 ± ±56.02	78.83 ± 53.51	0.0297
Total sperm count (mil/ ejaculate)			
Range	4-300	36–600	
Mean ± SD	86.4 ± 77.39	185.37 ± 137.82	<0.0001
Normal sperm morphology (%)			
Range	0–60	50–77	
Mean ± SD	34.83 ± 12.51	63.53 ± 6.46	<0.0001
Progressive sperm motility (%)			
Range	0–40	40–65	
Mean ± SD	23.17 ± 11.7	53.33 ± 7.23	<0.0001
PH	7.52±0.03	7.53±0.04	0.1407
Viscosity	0.7±.1	1.1±0.01	0.0001
Mean number of sperms per ml	14.48 ± 4.93 $\times 10^6$/ml	90.60 ± 39.22 $\times 10^6$/	< 0.001

Table 2. Comparison between conventional semen parameters among patients and controls. Data are presented as mean±SD, p value was set significant at less than <0.05

Comparison of semen parameters showed that the mean semen volume, in ml, was significantly lower in the infertile group (2.08 ± 0.84) than the fertile group (2.52 ± 0.53); (P= 0.0021. The mean of total sperm count (mil/ejaculate) is significantly lower in the infertile than the fertile group (86.4 ± 77.39 vs.185.37 ± 137.82,P<0.0001). Furthermore, when motility in seminogram of infertile and fertile group was compared, the infertile had a mean grade A motility of 11.84 ± 9.22 % and a sum of A+B motility of 26.77 ± 9.76 %, compared to 25.645 ± 6.33 and 56.7 ± 6.74 for the two types respectively in the control cases, (P <0.001). Similarly, normal sperm morphology was significantly lower in the infertile than the fertile group with a mean of (34.83 ± 12.51vas.63.53 ± 6.46, P<0.001).Semen of the infertile group showed a higher viscosity is thicker than in

Semen heavy metals level	Infertile patients (n=60)	Control group (n=48)	*P*-value
Low toxicity (25%)	3(5)	1(2.1)	0.7975
Medium toxicity	4(6.7)	0	0.2115
High toxicity	8(13.3)	0	0.0239
Total	15(25)	1(2.1)	0.0082

Table 3. Semen aflatoxin positive cases among infertile patients and control. Data are presented number (%),p value was set significant at less than <0.05

the controls (0.7±.1 vs. 1.1±0.01, P<0.0001, respectively), both groups showed almost similar semen PH (P=0.1407) as shown in table II.

Aflatoxins were detected in 25% (n=60) cases vs. 2.1% (n=48) case out of fertile male (P=0.008). The aflatoxin level in the seminal fluid among the infertile male was significantly higher compared to fertile men (P=0.0082). Among the infertile men, low aflatoxin level was reported in 3 cases (5%) compared to 1 (2.1%) cases in the fertile group, P= 0.7975, and medium toxicity was demonstrated in 4 (6.7%) cases among the infertile men, P=0.2115. The high level of toxicity was significantly higher among the fertile compared to infertile group (25% vs. 2.1%, P=0.0239) as shown in table III.

4. DISCUSSION

The most challenging aspects of infertility management are to identify the exact cause of infertility and to establish a plan of treatment options for the infertile couple. In general male infertility remains a challenge for primary health care providers.

In the present study, aflatoxin B1 (AFB1) was present in 25% of the semen of infertile patients, compared to 2.1% among the controls (P=0.0082). Another significant finding was abnormal semen parameters that included, severe reduction in sperm count, reduced motility, high percentage of abnormal morphology, and high viscosity in the semen of the infertile group(P<0.05) compared to the fertile group and the WHO reference values for normal semen parameters. A higher prevalence rate of aflatoxin in the semen and blood of infertile men was reported in a Nigerian study (37%)(12). Another study reported a prevalence a rate of 40% in infertile men compared to 8% of fertile [15]. In the same study, 50% of the infertile men with high aflatoxin semen levels also showed abnormalities in semen parameters.

This high prevalence of AFB1 in the semen of infertile group can be explained by the highest prevalence of aflatoxin B1 in nuts and seeds (82%), herbs and medicinal plants (29%), spices (40%), dried vegetables (25%), and cereal grains (21%) that are consumed by Egyptian people [16]. Although, this study did not try to identify the source of contamination of the infertile group by aflatoxin, a previous study found that 90% of aflatoxin in humans was due to dietary intake[17]. Hatem et al. reported high concentrations of aflatoxins in the serum (80%) and urine (46%) of Egyptian infants with kwashiorkor and marasmus [18]. Thus, early accumulation of aflatoxins in human systems, and particularly in the testes may perhaps explain the significant testicular damage reported in infertile men. Normally, ingested aflatoxin is metabolised at the liver to intermediate products, which are water-soluble so that it can easily be excreted by the kidney. When the liver function is jeopardized, the toxic material will accumulate in the different parts of the body leading to tissues damage. We did not assess the liver function in this study, a limitation which we believe that it would had yielded better results. These findings are in agreement with studies in animal models, which proved that; exposure to aflatoxins will lead to severe defect in morphology and physiology of spermatozoa [19]. A recent study on the effect of aflatoxins on ovarian follicular growth in rats demonstrated that, aflatoxins exert an atretogenic effect on growing and non-growing follicles associated with significant reduction in ovulatory follicles [20]. Even with the very low cut-off value for sperm morphology, as proposed in the new edition of the (WHO) on semen analysis, patients exposed to chronic aflatoxins developed a lower percentage of sperm morphology.

1AFB1 causes a wide range of toxico-pathological effect including acute toxicological effects, carcinogenicity, teratogenicity, genotoxicity, immunotoxicity and sometimes death[21]. AFB1 in human men is a cause of delayed testicular development, testicular degeneration, decreased reproductive potential, morphological regressive changes in the testis and impairment of Leydig cell function [22]. Damage to the chromosome and gene by aflatoxins were proved to be by coupling of aflatoxin to DNA, forming aflatoxin-DNA complex [23] which lead to mark abnormalities in human sperm[24].

The mechanism whereby these aflatoxins produce these deleterious effects is via generation of reactive species (free radicals). These species attack macromolecules including protein, DNA, lipid of the sperm and testicular tissues etc. causing cellular/tissue damage after depletion of natural antioxidants. Researchers have proved that the ability of human sperm to fertilize the ovum depends mainly on the sperm motility and the membrane integrity [25]. in our clinical practice, aflatoxins concentrations in the semen are not routinely performed. Supplementation of selenium and/ or vitamin E in idiopathic male infertility may improve the quality of semen and produces beneficial and protective effects on sperm motility [26]. The use of these potent anti-oxidants as an imperial treatment in idiopathic male infertility may be responsible neutralization of these circulating reactive species generated by aflatoxins.

The presence of aflatoxin in the semen of infertile men may be a cause of the abnormal semen parameters observed in this study. The shortcomings of this study are the relatively small sample. Furthermore, we did not investigate the liver function in the infertile subjects. However, we did not address the cause/effect relationship between aflatoxins and male infertility; these results are an invitation for further research.

5. CONCLUSION

This study determined a 25% prevalence rate of aflatoxins in the semen of men with idiopathic infertility; and toxin has been shown to produce deleterious effect on the testicular function evident by reduced semen parameters. We believed that aflatoxins are an important factor in

male infertility, yet a further study is of paramount importance to establish cause/effect relationship. Primarily, screening men with idiopathic infertility for AFB1 may be of help in diagnosis.

CONFLICT OF INTEREST: NONE DECLARED

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