ORIGINAL RESEARCH

Aflatoxin B1 as an endocrine disruptor among miller flour workers

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Abstract

Aim: Aflatoxin B1 has been stated to inhibit the function of different endocrine glands. This study was proposed to clarify the possible effects of aflatoxin B1 as an endocrine disruptor on pituitary gland, thyroid gland and gonads among miller flour workers, and to evaluate its effects on human male sexual function.

Methods: A case-control study was conducted in a flour mill in Helwan District Cairo, Egypt in 2019. The study included 42 exposed flour milling male workers from the grinding department which showed the highest level of aspergillus flavus in the air sampling of airborne fungi and 40 non-exposed males. Serumaflatoxin B1/albumin, luteinizing hormone, follicle stimulating hormone, testosterone, 17-beta-estradiol, free triiodothyronine, free thyroxin and thyroid stimulating hormone were measured for the studied groups.

Results: Sampling of airborne fungi revealed that aspergillus flavus and penicillum were the predominant fungal types in the flour mill. Indoor/Outdoor ratios for aspergillus flavus were ≥ 1 in all the locations indicating presence of indoor sources. Serum Aflatoxin B1/albumin, luteinizing hormone and follicle stimulating, the existence of various types of sexual disorders (decreased libido, impotence and premature ejaculation) were higher while testosterone was lower in the miller flour workers compared to non-exposed. However, there was no significant difference regarding 17-beta-estradioland thyroid hormone levels between both studied groups.

Conclusion: Aflatoxin B1creates possible human male reproductive health distresses in miller flour workers.

Keywords: *aflatoxin B1, Egyptianflour workers, LH and FSH, sexual disorders, testosterone, thyroid hormones.*

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Introduction

Worldwide, human fertility is deteriorating; a state that cannot be referred only to the increase in contraception usage (1). Many environmental factors, industrial and occupational compounds, dietary contaminants, lifestyle factors and medications were suggested to be other causes to this deterioration (1).

Male infertility causes may be pre testicular, testicular and post testicular. The pre testicular and the testicular causes are chiefly endocrine disorders that originate from the hypothalamic-pituitary-gonadal axis that have opposing effects on spermatogenesis (2).

Male reproduction is controlled by the hypothalamo-hypophyseal testicular axis: hypothalamic gonadotropin releasing hormone, pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH) and the gonadal steroid, principally, testosterone. It was proved that thyroid hormones have a changeable effect on this axis and thus affect the sexual and spermatogenic function of man (3). Effects of thyroid hormones occur through binding to certain thyroid hormone receptors which are extensively spread in the testis (4).

Endocrine disrupting chemicals (EDCs) may be of synthetic (pesticides, industrial chemicals, bisphenols) or natural origin (mycotoxins, phytoestrogens). Some mycotoxins can act as probable endocrine disruptors and cause changes in hormone production (5). Endocrine disruptors may simulate the action of sex hormones, affect reproduction (6), cause reproductive anomalies (morphological and functional gonadal dysfunction, e.g. infertility and decreased libido) and congenital malformations (altered embryonic and foetal intrauterine development) (7).

Egypt is one of the countries with high wheat consumption (8). Fungi can produce varied types of mycotoxins under environmental conditions which are favourable to growth.

Aflatoxins are naturally occurring mycotoxins produced by certain fungi, mainly aspergillus flavus and aspergillus parasiticus. Aflatoxins B1 (AFB1) is one of the main aflatoxin types (9).

AFB1 has been stated to inhibit the function of different endocrine glands by disturbing the enzymes and its substrate that are responsible for the synthesis of different hormones (10). Aflatoxins have the ability to generate hormonal dysfunction inducing cell toxicity which directly affects reproduction (11). Previous studies stated that AFB1 disturbs the hypothalamo-pituitary testicular axis resulting in production of malfunctioning spermatozoa (12,13). Uriah and his colleagues (14), proved that aflatoxin levels in the blood and semen of infertile Nigerians men were significantly higher than in the fertile men, suggesting that aflatoxin might be an influential factor in occurrence of men infertility. AFB1 lower sensitivity of thyroid receptors by enhanced generation of reactive oxygen species, aggravating lipid peroxidation concentrations (15).

Mycotoxins could be raised in animal and human biological fluids after feeding of contaminated food products. However, nowadays contamination through inhalation of mycotoxins in indoor air has been taken in consideration (16).

This study was proposed to clarify the possible effects of aflatoxin B1 as an endocrine disruptor on pituitary gland, thyroid gland and gonads among miller flour workers, and to evaluate its effects on human male sexual function.

Methods

Study design

This was a case-control study. The exposed workers were considered as cases compared to the non-exposed subjects (controls).
The variable fertility/sub-fertility in this study was measured through estimation of the sex hormones as high LH and FSH and low testosterone and rate of occurrence of sexual male function disorders among the exposed workers compared to their non-exposed can affect the fertility of the exposed workers.

Study population and sampling

This study included all the miller flour exposed male workers (42 workers) from the grinding department (which showed the highest level of aspergillus flavus in the air sampling of airborne fungi). Forty male non exposed subjects were included in the study (they were all the available employees working in the area surrounding the flour mill).

Data collection

Written informed consent was obtained from all the included subjects. Questionnaire was filled during personal interview with the participating groups. The questionnaire included (personal data, detailed occupational history, marital, sexual and medical history, types and use of personal protective equipment). Available personal protective equipment included masks, protective goggles and gowns.
Exclusion criteria were obesity, history of diabetes, hypertension and thyroid diseases, which may be considered differential causes for infertility.

Sampling of airborne fungi

The samples were taken during the normal working days, between 9.00 am to 2.00 pm to determine peak exposures inside the flour mill. The air sampler was positioned at a height of ~ 1.5 m (breathing zone) above the floor level in the middle of the sampling location. The control(comparison) samples were taken 10 m outside the building.
Andersen one-stage viable cascade impact or sampler (TE-10-160, Tisch Environmental Cleves, OH, USA) was used. It collects particles with aerodynamic diameter of < 2.5 µm. Particles < 2.5 µm penetrate deeply into lungs. The sampler was operated at flow rate of 28.3 L/min for 5 min. Malt extract agar (MEA) were used to collect airborne fungi (BD BioSciences, Sparks, Maryland, USA). Three consecutive samples were taken during each sampling event (3 plates/location).
Fungal plates were incubated at 28 °C for 5-7 days and checked daily. The resultant colonies were counted and positive hole correction was conducted on all counts prior to the calculation of the colony forming units per cubic meter of air (CFU/m^3) (17).
Fungal isolates were purified and identified by direct observation on the basis of micro and macro morphological features. Identification was performed on the basis of reverse and surface coloration of colonies on Sabouraud dextrose agar, CzapekDoxagar, potato dextrose agar and malt extract agar. Fungal isolates were identified to the genus or species level (18).

Laboratory investigations

-The blood samples were collected in sterile dry tubes, left to clot for 30 min and then centrifuged at 3000 r/min for 10 min. The separated sera were kept at -20 ˚C for the laboratory investigations.

-AFB1 and serum Alb:

- Aflatoxin B1 was firstly extracted using EASIEXTRACT1 aflatoxin immune affinity column (Scotland). AFB1 concentrations of the samples were analyzed by micro-titer plate enzyme-linked immune-sorbent assay

(ELISA) method using RIDAS-CREEN1AFB1 30/15 ELISA, made in Germany.

- Serum albumin (Alb) was determined by colorimetric method according to Doumas and Biggs (19).

-Serum concentrations of LH,FSH, testosterone, free triiodothyronine (fT3), free thyroxin (fT4) and thyroid stimulating hormone (TSH) were measured using ELISA Kit by DRG International, Inc., (USA) at the research laboratory, 17-beta-estradiol (E2) using kit by Biosource.

Ethical approval number (10142) was obtained from the Research Ethics Committee of the National Research Centre, Egypt, before the beginning of the study.

Data analysis

Statistical analysis was done through SPSS package version 20. Quantitative data were expressed as mean ± SD. Two independent sample T-test and Chi-square test were used to assess statistical differences in the quantitative and qualitative data (for distribution of sexual disorders among the studied groups) respectively between the exposed and the non-exposed groups. Pearson's correlation coefficient was calculated for exposure duration, aflatoxin B1 and studied hormones among the exposed workers. P-values were two-tailed and considered statistically significant at ≤0.05.

Results

Both studied groups were between 40 to 50 years, with mean age 45 ± 8.9 years for the exposed workers and 44± 9.2 years for the non-exposed group; without significant difference. There was no significant difference between both studied groups regarding smoking habits; number of smokers was 26 among the exposed group and 24 among the non-exposed group. The mean of duration of exposure of the miller flour workers was 15 ± 5.2 years. None of the workers in the flour mill used personal protective equipments.

Penicillium and Aspergillus were the common airborne fungi in the flourmill. Penicillium and Aspergillus flavus concentrations ranged within39– 577 CFU/m3 and 12– 205 CFU/m3, respectively. Penicillium and Aspergillus flavus were found in the highest concentrations in the grinding unit. Aspergillus niger concentrations ranged from 19– 180 CFU/m3, with the highest concentration found in garbling unit.

Table 1 shows Indoor/outdoor (I/O) ratio, "a relative standard" used to document the presence or absence of indoor biologically derived contamination and differences between sampling sites (20). In the present study, I/O ratios for penicillium and aspergillus niger were ≤ 1 in almost all locations; suggesting that outdoor air was the main contaminant source. However, I/O ratios for aspergillus flavus were ≥ 1 in all the locations indicating presence of indoor sources

Table 1. I/O ratios of the common airborne fungi at the flourmill units

Locations	*Penicillium* species	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus* species*
Storage	0.07	6.68	0.5	-
Garbling	0.2	6.67	0.75	0.42

Grinding	1.12	17.83	0.12	0.42
Packaging	0.26	1.86	1.16	0.57

*Aspergillus species include: *Aspergillus parasiticus*, *Aspergillus terreus* and *Aspergillus ochraceus*.

Table 2 shows that AFB1-Albumin (AFB1-Alb) level among the exposed group was significantly higher compared to the non-exposed. LH and FSH were significantly higher and testosterone was significantly lower among the exposed workers compared to the non-exposed. There was no significant difference regarding E2 and the thyroid hormones between the two studied groups. Data in table 3 shows that among the exposed workers, AFB1/Alb is significantly positively correlated with LH and negatively correlated withfT4 & fT3. fT4 is significantly negatively correlated with the duration of exposure. TSH was significantly negatively correlated with LH and FSH, and positively correlated with testosterone. LH was positively correlated with FSH on one side and negatively correlated with testosterone on the other side. Twenty-five percent (10/40) of the non-exposed and 45.2% (19/42)of the exposed group complain of sexual disorders. Fifteen percent (6/40) of the non-exposed versus 7.1% (3/42) of the exposed workers complained of one sexual disorder. While 10% (4/40) of non-exposed versus 38.1% (16/42) of the exposed workers complained of more than one sexual disorder. Table 4 shows the distribution of various types of sexual disorders (decreased libido, impotence, premature ejaculation) which was higher in the exposed workers.

Table 2. Comparison of AFB1-Albumin (AFB1-Alb) level, the male sex hormones, E2 and thyroid hormones between the two studied groups

	Non-exposed (=40)		Exposed (=42)		Independent t-test	
	Mean	**SD**	**Mean**	**SD**	**t-test**	**P-value**
AFB1/ALB ng/g	0.04	0.01	0.06	0.02	4.658	P< 0.001
LH (3-12mIU/ml)	5.763	.1469	7.542	.3271	4.960	P< 0.001
FSH (2-10mIU/ml)	6.442	.2644	30.542	3.9841	6.036	P< 0.001
Testosterone (0.083 – 16ng/ml)	5.5268	.19092	4.0593	.47891	2.846	P= 0.006
17β-estradiol E2 (11.2-43.2pg/ml)	29.6789	1.96325	31.8016	2.70151	0.636	0.527
fT4 (0.93-1.7ng/dl)	1.33	0.17	1.32	0.22	1.52	0.880
fT3 (2-4.4pg/ml)	2.75	0.36	2.92	0.60	1.51	0.135
TSH (0.5-8.9uIU/ml)	1.89	0.55	2.11	0.67	1.59	0.117

AFB1-Albumin (AFB1-Alb), LH= luteinizing hormone, FSH= follicle stimulating hormone, E2 =17β-estradiol, fT4= free thyroxin, fT3= free triiodothyronine, TSH= thyroid stimulating hormone.

Table 3. Relation between exposure duration, aflatoxin B1 and studied hormones among the exposed workers

		Exposure duration	AFB1/ALB	LH	FSH	Testosterone	E2
Exposure duration	r=	-	0.2	-.121	-.053	.200	.060
LH	r=	-.121	0.3*	-	.779**	-.322*	-.067
FSH	r=	-.053	0.1	.779**	-	-.294	-.110
Testosterone	r=	.200	-0.1	-.322*	-.294	-	-.051
E2	r=	.060	-0.03	-.067	-.110	-.051	-
fT4	r=	-.336*	-0.3*	.073	.097	-.020	.163
fT3	r=	-.066	-0.3*	.096	.004	-.050	.088
TSH	r=	-.063	-0.01	-.507**	-.334*	.342*	-.161

** p<0.01, * p<0.05, r= Pearson's correlation;AFB1-Albumin (AFB1-Alb), LH= luteinizing hormone, FSH= follicle stimulating hormone, E2 =17β-estradiol, fT4= free thyroxine, fT3= free triiodothyronine, TSH= thyroid stimulating hormone.

Table 4. Distribution of sexual disorders among the studied groups

Sexual symptoms	Non-exposed (40)		Exposed (42)		Chi-square
	Number	%	Number	%	P-value
Decreased libido	3	7.5	10	23.8	0.04 *
Impotence	5	12.5	12	28.6	0.07
Premature ejaculation)	7	17.5	17	40.5	0.02*
Infertility	2	5	3	7.1	0.6

* P<0.05.

Discussion

In the present study, sampling of airborne fungi revealed that aspergillus flavus & penicillum were the predominant fungal types in the flour mill. Indoor/Outdoor ratios for aspergillus flavus were ≥ 1 in all the locations indicating presence of indoor sources. Serum AFB1/Alb, LH and FSH, existence of various sexual disorders (decreased libido, impotence and premature ejaculation) were higher while testosterone was lower in the miller flour workers compared to non-exposed. However, there was no significant difference regarding 17-beta-estradiol E2 and thyroid hormone levels between both studied groups.

AFB1/Alb is significantly positively correlated with LH and negatively correlated with fT4 & fT3. fT4 is significantly negatively correlated with the duration of exposure.

The results in the present study agree with Awad study (21) which found that Aspergillus and Penicillium were the dominant airborne fungi in flourmill buildings.

The concentrations of Aspergillus flavus inside the different flour mill units exceeded outdoor ones. The dominance of Aspergillus flavus is an indication of inadequate storage conditions and high water content of grains (21). Contamination of different grains by Aspergillus niger, Aspergillus flavus and Fusariumoxysporium occurred due to poor environmental conditions during pre and postharvest of grains (22). In the present study, indoor/outdoor (I/O) ratios of Penicillium, Aspergillus niger and other Aspergillus species almost did not exceed 1, meaning that outdoor was the main source. However, I/O ratio of Aspergillus flavus exceeded 1, reached 17.83 (in grinding) indicating the presence of inside generative sources (23).

AFB1, have great effect on the endocrine glands and reproductive system both in humans and in experimental animals. However, literature on the effect of aflatoxin on human reproduction is scarce (24).

Serum AFB1-Albadduct level was significantly higher among the miller flour workers compared to the non-exposed in the present study. AFB1-Alb level detection in serum is a reliable indicator of long-term exposure to aflatoxin (25). So, the rise of AFB1-Alb level among the workers could be attributed to their occupational exposure to relatively high concentrations of aspergillus flavus through inhalation by handling flour dust which represents an additional exposure risk to those subjects than the general population, which was confirmed by the high I/O ratio of Aspergillus flavus.

The present study showed decreased serum testosterone and increased serum level of FSH and LH among the miller flour workers compared to the non-exposed. Moreover, there is positive correlation between LH &AFB1/ALB in the exposed group. These findings may be due to increase level of AFB1-Alb among the exposed workers than the non-exposed, which could be due to the high concentration of aspergillus flavus in the air environment of the flour mill in the present study.

Previous studies (26,27) showed that concentrations of serum FSH, LH and testosterone were reduced in AFB1 treated rats. Another study (28) found similar results in male chicken fed on different concentrations of dietary aflatoxin. Results showed decreased serum testosterone levels and LH in the aflatoxin treated groups compared to the control group. Another author (29) administered AFB1orally in male rats for 48 days at different doses. The concentrations of serum LH and testosterone were lower, but on the other hand serum FSH was higher in the treated groups.

After the administration of different doses of AFB1, the concentration of serum testosterone was significantly reduced, in a dose-dependent manner in rabbit (30); in Japanese breed quails (31); in white leghorn male chicken (32) and in goats (33).

The diversity of results of various experimental animal studies could be due to species variances or due to difference in route of exposure, potency or the dose of AFB1and the duration of exposure.

In a previous study (34), the serum testosterone concentration was significantly lower while the levels of serum FSH and LH increased significantly in adult rats exposed to AFB1 compared to non-exposed. These findings agreed with the results of the present study.

Verma and his colleague (35) supported that reduced serum testosterone concentration is attributed to mitochondria dysfunction, to inhibition in protein synthesis or enzyme activity or to membrane changes of leydig cells. They added that increased level of LH along with decreased level of serum testosterone in experimental rats exposed to AFB1 reflect decreased steroidogenic ability of the testes suggesting permanent changes in leydig cell function.

The degenerative effect of the aflatoxin on germinal epithelium of the seminiferous tubules would breakout into sertoli cells, leading to decrease in inhibin B1 level thus reducing its inhibitory effect on secretion of FSH leading to its elevation (36).

Direct effect of AFB1on leydig cells and sertoli cells in the testes leading to reduction of the gonadal hormones; testosterone and estradiol may be due to the action of AFB1 on binding of DNA to form complexes and inhibition of nucleic acid synthesis (32).

In the current study, 45.2% of the exposed group versus 25% of the non-exposed group complained of sexual disorders. The distribution of different types of sexual disorders (decreased libido, impotence, premature ejaculation) was higher among the miller flour workers compared to non-exposed. This might be due to increase of (AFB1-Alb) level in the exposed group than in the non-exposed as some mycotoxins can act as probable endocrine disruptors and cause changes in hormone production (5) and can cause reproductive anomalies (morphological and functional gonadal dysfunction, e.g. infertility and decreased libido) (7). Also, the decrease of testosterone in the exposed group might be the cause of decreased libido, and potency in this group, as testosterone is necessary to maintain male secondary sex characteristics, libido, and probably potency. Thus patients with endocrine abnormalities may present with variety of symptoms, elevated levels of the gonadotropins, FSH and LH in the presence of decreased testosterone levels indicating primary testicular dysfunction (37), which agreed with the results of the present study.

In the present study, although there was no significant difference between the exposed and the non-exposed groups regarding the levels of the thyroid hormones, yet, there was negative correlation between fT4 and duration of exposure, and between fT3, fT4 and AFB1-Alb. These findings might suggest thyroid gland affection by aflatoxin on the long run.

Moreover, there was a negative correlation between TSH and both LH, FSH, and positive correlation between TSH and testosterone, which means that decrease of TSH level occurred with lowering of testosterone and elevating LH & FSH levels indicating intact hypothalamo-pituitary-thyroid axis.

Limitation of the study

Information on sexual health was assessed using self-reporting which is a source of information bias. Further studies are needed to be done on a larger scale.

Conclusion

Our results showed that AFB1 causes alterations in the serum concentrations of the gonadotropic (FSH and LH), as well as gonadal (testosterone) hormones in the form of significant increase in the serum concentrations of LH and FSH, as well as significant decrease in testosterone levels among exposed workers. The lowered levels of testosterone with elevated levels of FSH and LH indicate intact pituitary testicular axis in AFB1 exposed workers. These findings may confirm the ability of AFB1as endocrine disruptor to affect human male reproductive health. That is why it is highly recommended to estimate the levels of both gonadotropic (FSH and LH)

hormones periodically in exposed workers to pick up any early changes in their levels.

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