SEX HORMONE PROFILE AND PERCEIVED SEXUAL FUNCTIONS IN MALE VAT DYE WORKERS

Supported by tetfund

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Received: March 16, 2020 **Accepted:** May 04, 2020

Abstract: Some chemicals and occupational exposures have been implicated in reproductive dysfunctions. Furthermore, certain textile dyes have been associated with testicular toxicity and infertility. It is not known if such exists, among vat dye workers in Abeokuta, Southwest, Nigeria. To determine the effects of exposure to vat dye on sexual functions and levels of sex hormones among the male, thirteen dye workers, age ranging from 19 to 53 with minimum of two years' duration of exposure were aged matched with nine unexposed male participants. Socio-demographic, occupational, sexual function and lifestyle characteristics were obtained from interviewer-administered semi structured questionnaires. LH, FSH, Prolactin and Testosterone were determined by enzyme immunoassay, using kits purchased from Immunometrics, United Kingdom. Results were expressed as mean (SD) for unskewed and mean rank for skewed data. P values ≤ 0.05 were considered significant. The mean rank of the levels of Testosterone in the exposed (12.58) was significantly higher than that in the unexposed (5.57) ($P<0.001$). Serum levels of LH, FSH and Prolactin were similar in the two groups ($P>0.05$). Erectile dysfunction was observed in 8.3%, ejaculation problem in 72.7% and lack of sexual interest in 30.8% of the exposed population, similar to that of the control subjects. Male Participants occupationally exposed to vat dye experience raised level of serum Testosterone which increases with increased duration of exposure. An imbalance in hormone level(s) can result in adverse reproductive outcome. Erectile dysfunction, ejaculation problem and lack of sexual interest among dye workers cannot be related to exposure to vat dye.

Keywords: Exposure, male, occupational, sexual function, testosterone, vat dye

Introduction

In recent decades, a downward trend in reproductive activities of male and female subjects has been reported which is thought to be associated with chemical and occupational exposures (Burdorf *et al.,* 2006; Golden *et al.,* 1999; Kumar, 2004; UNEP and WHO, 2013). These effects include decreased sperm count and quality (Golden *et al.,* 1999; Sheiner *et al.,* 2003) alterations in male and female reproductive hormone levels (Padungtod *et al.,* 1998), menstrual cycle disorders (Eskenazi *et al.,* 2002), reduction in fertility (Kumar, 2004), longer time to pregnancy (Snijder *et al.* 2012), increased risk of spontaneous abortion (Xu *et al.,* 1998) and increased risk of pre-term delivery (Kumar, 2004). Examples of the chemicals that have been implicated include heavy metals such as lead and cadmium; organic solvents such as glycol ethers, toluene, and vinyl chloride; pesticides and herbicides such as ethylene dibromide; dyes; sterilants; anesthetic gases and anticancer drugs used in health care (Lawson *et al*., 2003; Rachootin and Olsen 1983).

More specifically, in 2001, Cherry and his team reported that occupational exposure to organic solvents constitutes a hazard for male fertility. In 2005, Park *et al*. (2005) reported a case of erectile dysfunction associated with chronic methyl bromide intoxication. Furthermore, Li *et al.* (2010) reported that BPA-exposed workers had consistently higher risk of male sexual dysfunction across all domains of male sexual function (increased risk of reduced sexual desire than the unexposed workers, erectile difficulty and ejaculation difficulty).

The male reproductive system is vulnerable to the effects of these chemicals probably because sensitive effects take place during spermatogenesis and the persistent environmental pollutants and /or physical factors may affect some of these events to some extent (Kumar, 2004). Also, some of these chemicals effect induction of ROS, lipid peroxidation, and apoptosis of spermatocytes (Al-Mashhedy, 2013; Jeng, 2014). Reproductive outcomes in males include sperm abnormality, hyperoestrogenism, impotence, infertility and or increased spontaneous abortion rate in wives of workers.

Regarding dyes and reproductive effects, Fernandes *et al*. (2015) reported harmful activity of Disperse Red1 (a textile dye and an azo class of dye) on reproductive health of male mouse. Gray and Ostby (1993) reported that testes of male offspring of mice exposed to benzidine-based dyes (Congo red, diamine blue and chlorazol black E) were observed to be small and contained tubules completely devoid of germ cells. They reported alteration in testicular development and hypospermatogenesis in mice during adulthood. Nevertheless, information is lacking particularly in Africans and in males, on the reproductive health effects of textile dye workers. Importantly, the class of dye used in "Abeokuta", Ogun State, South West, Nigeria, where this study was conducted is vat dye, and it is poorly characterized with limited data and their implications, particularly on the reproductive system with far reaching consequences. This study was therefore carried out to investigate the reproductive health of male vat dye workers. It assessed the sex hormone profile and sexual functions of male vat dye workers.

Methods employed in the assessment of male reproductive capacity in epidemiologic studies have included the use of questionnaires to elicit bio data, reproductive history and sexual function, lifestyle characteristics and occupational history; reproductive hormone assay (serum FSH, LH, prolactin and testosterone) (Golden *et al.,* 1999; Ibrahim *et al*., 2011; Padungtod, 1998 and Sakr *et al.,* 2010). More frequently, seminal fluid analysis is done to investigate sperm concentration, percentage of normal morphology, motility, liquefaction time, pH, and viability as well as fructose and zinc concentrations (Golden *et al.,* 1999; Kumar, 2004; Yucra *et al.*, 2006).

Materials and Methods

Design/occupation/population

This cross sectional study is the male aspect of a doctoral thesis which focused on reproductive characteristics of vat dyes workers located in "Itoku", "Abeokuta", Southwest, Nigeria. The study was conducted between the years 2010 and 2013 by Soyinka (2017). Textile dyeing is an occupation that

is indigenous to the people of Abeokuta. It used to be an occupation involving male and female subjects. About a hundred of them comprising of males and females registered with the work association during this period. In the recent, the number of male subjects involved in this small scale business has drastically reduced for unknown reasons. Purposive sampling technique was utilized, whereby; the total population of dye workers was invited to participate after they have been briefed on the aim, objectives and the significance of the study.

Inclusion and exclusion criteria

We recruited only those within the reproductive age who have worked with dyes for a minimum of two years, within the occupational setting, age between 19 and 53 years and male individuals who gave their consent. We excluded interested participants that could not be traced to their work location, those who either refused or were not available for blood sampling.

Community entry

Community entry to this target population was through the Leader of the work association. She further informed the Executive members. An arrangement was then made for the Principal Investigator and the interviewer to attend one of the association meetings where the aim, objectives and significance of the study was enumerated.

Recruitment

Thirteen male participants exposed to vat dyes met the inclusion criteria. Nine unexposed male participants served as controls. They were residents of Abeokuta and were recruited from among the ward attendants at the State Hospital, "Ijaiye" and a private group of schools at "Asero".

Interview and measurements

An interviewer-administered semi structured questionnaire written in English language was used to obtain information on socio-demographic, occupational and reproductive characteristics. The questionnaire was interpreted to the local language which was "Yoruba" and was so administered by a trained male interviewer. The socio-demographic aspect of the questionnaire included the age, sex, weight, height and marital status. The height and weight were measured as described by Sánchez-García *et al*. (2007) with the participants wearing light clothes without shoes. The weight was measured in kilograms with a bathroom scale (Hanson, China), the height was measured in "cm" using a long calibrated meter rule, with a bar placed on the head for accurate measurement and this was converted to meter, by dividing by 100. The major aspect of occupational history reported was duration of exposure (in years). With respect to male reproductive health, sexual functions were based on erection, ejaculation and sexual desire or interest. For erection, participants answered yes or no, to the question: have you ever gone through a period of several months when you had trouble getting or keeping erection? For ejaculation, they responded yes or no, to the question: do you get satisfactory ejaculation of sperm during intercourse and for sexual desire, have you ever gone through a period of several months when you had little interest in sex?

Blood sampling

About 10 ml of venous blood was collected from the ante-cubital vein with pyrogen free syringes and needles (Lifesign, London, Hypoject -IV) and the blood was dispensed into two 5 ml plain bottles. The blood samples were transported in ice bags and centrifuged at 700 x g for 5 min. The serum samples were separated from the cells and each subject's sample was stored as three aliquots at -20^{o}C for the various analyses of the entire study. Assays of Serum Luteinizing Hormone, Follicle Stimulating Hormone, Prolactin, and Testosterone were performed by enzyme immunoassay (EIA) methods using kits purchased from Immunometrics, United Kingdom using one of the aliquots. The procedures for the assays were carried out according to the instructions in the manual inserted in the kits.

Statistical analysis

The statistical Package for the Social Sciences, version 16.0 was used for data entry, while both versions 16 and 20 were used for the statistical analyses. Descriptive statistics, such as frequencies and percentages were generated for categorical variables, while means and standard deviations were for quantitative variables. Inferential statistics such as chi-square test and T-test were employed to test for associations of categorical variables and differences in mean values respectively. P-value less than or equal to 0.05 was considered to be significant.

Ethics

Ethical clearance and approval were obtained from the Scientific and Ethical Review Committee of Olabisi Onabanjo University Teaching Hospital, "Sagamu", Ogun State. Consent Paper was signed by all the participants.

Results and Discussion

In recent times, chemical and occupational exposures have been reported to cause divers health challenges among which are reproductive health disorders. This study was embarked upon to determine the effect of occupational exposure to vat dye on male sexual functions and levels of sex hormone among male dye workers in Abeokuta, South West, Nigeria.

The study comprises of twenty - two participants altogether, thirteen (59.0%) of which were exposed participants with mean (SD) age 32.3 ±8.25 years and nine (41.0%) control participants, with mean (SD) age 32.0 ±11.2 (years). Both groups were matched for age and BMI. About half of the exposed participants did not go beyond primary education. Very few of them read beyond secondary school. Only about half of them were married as shown in Table 1.

Table 1: Demographic characteristics of male textile vat dye workers and the unexposed participants

Parameter	Exposed	Unexposed	t/Z	P-value
	n [Frequency (%)]			
Sex				
Male	13 (59.0)	9 (41.0)		0.11
Educational background				
No formal education/primary	5(50.0)	1(11.1)		0.27
Secondary	4(40.9)	4 (44.4)		
Post-secondary	1(10.0)	4(44.4)		
NR	3	0		
Marital status				
Married	7(53.8)	4(44.4)		1.00
Singles/others	6(46.2)	5(55.6)		
	Mean (SD)		t	
Mean (SD) Age	13(32.3±8.25)	9(32.0±11.2)	0.07	0.94
	Mean Rank		Z	
BMI	9(9.22)	7(7.57)	0.69	0.49

t=t value; P = probability, n=Sample number, BMI= Body Mass Index, Z= Z value, SD =Standard deviation, %= Percentage, BMI= Body mass index; NR= Non respondents

Table 2: Male serum hormone levels in the vat dye exposed workers and the unexposed

Sex Hormone	Mean Rank		Z-value	p-value
	Exposed n=12¥	Unexposed n=9		
LH (IU/L)	12.54	8.94	1.32	0.19
FSH (IU/L)	12.00	9.67	0.86	0.39
Prolactin (mIU/L)	12.25	9.33	1.09	0.28
Testosterone (nmol/L)	12.58	5.57	2.67	0.00*

LH=luteinizing hormone, FSH =Follicle stimulating hormone; Results are expressed as mean rank; n =number of subjects used; P= probability; Z-test statistics, *statistically significant value, ¥ shows 1NR (Non respondent among the exposed)

Levels of prolactin and the gonadotrophic hormones were seen to be similar in both the exposed and the unexposed. Testosterone was however significantly higher in the exposed than the unexposed P<0.001 as shown in Table 2. This increase may be linked to the occupational exposure to vat dyes. Our comparisons were made with results of different exposures as we could not get literatures on dye exposure and hormone levels. Similar to our result, Meeker *et al.* (2010), in their study of environmental exposure to metals such as cadmium, copper and lead observed raised level of testosterone. Some of these heavy metals have been however reported to be raised in textile materials, particularly in dyes (Tuzen *et al.*, 2008; Sani *et al.*, 2018). It is therefore a possibility, that, the raised testosterone level was a contribution from heavy metals in these dyes. One of our limitations was that, we did not determine the levels of heavy metals in this study; hence we cannot relate the raised level of testosterone to the presence of heavy metals in vat dye occupational exposure. This is thus recommended in further studies.

This increase in testosterone level can also be explained from the interaction of the vat dye, with receptor binding site, thus disturbing the hormone-receptor interaction that would have ordinarily occurred (Pollack *et al.*, 2011). In contrast to our findings, Eldesouki (2013) reported a significant lower value of testosterone and higher values of LH and FSH in males who were occupationally exposed to volatile organic compounds. Luderer *et al.* (2004) in the study of solvent exposure (total solvents, chlorinated and aromatic solvents) did not find a significant association of solvents with testosterone, LH and FSH levels. Yucra *et al.* (2006) reported decreased testosterone level in exposure to pesticides among male subjects. Padungtod *et al.* (1998) on the other hand, did not find any significant association between occupational pesticide (ethylparathion and methamidophos) exposure and testosterone, but elevation in FSH and LH. Similar to this study, Nagata *et al.* (2015), observed an increase in testosterone level in premenopausal women with exposure to permanent hair dyes for ten or more years compared with the control. While we worked with textile dyes, they studied hair dyes. We worked with males, while they worked with women. We however, observed, that, the level of testosterone increases with duration of exposure as seen in Table 3. In hormone regulation, higher levels of gonadal hormone could either originate from hypothalamus-anterior pituitary gland or from the gonad itself, due to excessive synthesis and secretion. Our finding did not show any significant changes in the levels of both FSH and LH hence it could be said that the observed increased levels of the gonadal hormone are probably from the testicular glands and did not originate from the hypothalamus-pituitary regulatory axis.

Table 3: Correlation studies of length of time in occupation and sex hormone levels in male dye-exposed population

	Sex Hormone	r	P-value
Male(n=13)			
	LH	-0.21	0.52
	FSH	-0.30	0.34
	Prolactin	0.41	0.18
	Testosterone	0.57	0.05*

r= correlation coefficient; p= probability; *represents significant values at p≤ 0.05; LH =luitenizing hormone, FSH = Follicle stimulating hormone at P=0.05

Table 4: Sexual Dysfunction among Male Vat Dye Exposed and Unexposed Subjects

Erection Problem	n=13	Exposed (%)	n=7	Unexposed (%)	P-value
Yes	1	8.3	0	0.0	1.00
No	11	91.7	7	100.0	
NR	1		0		
Ejaculation problem					
Yes	8	72.7	3	75.0	1.00
No	3	27.3	1	25.0	
NR	2		3		
Lack of sexual interest					
Yes	4	30.8	1	16.7	1.00
No	9	69.2	5	83.3	
NR	0		1		

n=sample number; NR=non respondent

Only few of the exposed participants experienced erection problem while none of the unexposed participants experienced such. The proportion with ejaculation problem was similarly high in the two groups. About thirty percent reported lack of sexual interest amidst the exposed population. This was similar to the sixteen percent who reported same among the unexposed as shown in Table 4.

Increased testosterone has been associated with increased sexual activities such as ejaculation, increased sexual desire and erectile function in men and vice versa (Corona *et al.*, 2008; Snyder *et al.*, 2016). In this study we assess sexual function with regards to erection, ejaculation problem and lack of interest in sex.

Male participants occupationally exposed to vat dyes did not experience sexual dysfunction such as erectile dysfunction, ejaculatory failure and lack of sexual interest that can be related to vat dye exposure. On the contrary, Park and his team documented erectile dysfunction in a 45 year old fumigation department worker following chronic exposure to Methyl bromide. Also, Li *et al.* (2010) reported a significantly increased risk of reduced sexual desire, erectile difficulty, and ejaculation difficulty and reduced satisfaction with sex life among workers exposed to BPA.

Some studies have shown that levels of circulating testosterone in individuals developing prostate cancer are higher than in controls, though not without controversies (Slater and Oliver 2000). Further studies should therefore investigate risk of prostate cancers among male vat dye workers, more importantly because some forms of cancers have earlier been associated with dyes (Mastrangelo *et al.*, 2002).

Conclusion

The study has shown that exposure to vat dye within the limits of exposure among male vat dye workers in Abeokuta resulted in raised level of testosterone which increases with duration of exposure. An imbalance in hormone level(s) can result in adverse reproductive outcome. Regular evaluation of levels of testosterone in male dye workers is thus recommended. Sexual dysfunction among the dye workers is not related to their occupation.

Acknowledgements

This is to acknowledge the Director of former Center for Research in Reproductive Health (CRRH), Sagamu, Ogun State, Prof. O. A. Dada for the provision of facility and laboratory instrument. Support from members of staff of CRRH is also appreciated. The contribution of Dr. K. S. Oritogun in the area of data analysis is acknowledged.

Conflict of Interest

Authors declare that there is no conflict of interest related to this study.

References

Al-Mashhedy LAM 2013. Toxicity Assessment of textile dyes via oxidative stress hypothesis for Iraqi textile workers. *Int. J. Pharma. Bio. Sci.*, 4(4)(B): 577 – 587.

Burdorf A, Figa-Talamanca ITK &Thulstrup AM 2006. In-depth reviews. Effects of occupational exposure on the reproductive system: core evidence and practical implications. *Occupational Medicine*, 56: 516-520.

Cherry N, Labrèche F, Collins J & Tulandi T 2001. Occupational exposure to solvents and male infertility. *Occup Environ Med*., 58: 635–640.

Corona G, Jannini EA, Mannucci E, Fisher AD, Lotti F, Petrone L, Balercia G, Bandini E, Chiarini V, Forti G & Maggi M 2008. Different testosterone levels are associated with ejaculatory dysfunction. *The J. Sexual Med.*, 5(8): 1991-8.

Eldesouki MA, Sharaf NE, Abdel Shakour AA, Mohamed MS, Hussein AS & Hasani I W 2013. Study of the effect of occupational exposure to volatile organic compounds (VOC's) on male reproductive hormones. *World J. Med. Sci.*, 8(1): 06-12.

Eskenazi B, Warner M, Mocarelli P, Samuels S, Needham LL, Patterson DG Jr., Lippman S, Vercellini P, Gerthoux PM, Brambilla P & Olive D 2002. Serum dioxin Concentrations and menstrual cycle characteristics. *Am. J. Epidemiol.*, 156(4): 383-92.

Fernandes FH, Bustos-Obregon E & Salvadori DM 2015. Disperse Red 1 (Textile dye) induces cytotoxic and genotoxic effects in mouse germ cells. *Reproductive Toxicology*, 53: 75-81.

Golden AL, Moline JM & Bar-Chama N 1999. Male reproduction and environmental and Occupational exposures: A review of epidemiologic methods. *Salud Publical deMexico*, 41(2): S93 - S105.

Gray LE JR & Ostby JS 1993. With technical assistance. ER Marshall, ManTech Inc. 1993. The effects of prenatal administration of azo dyes on testicular development in the mouse: A structure activity profile of dyes derived from benzidine, dimethylbenzidine, or dimethoxybenzidine. *Toxicological Sciences*, 20(2): 177–183, https://doi.org/10.1093/toxsci/20.2.177

Ibrahim KS, Amer NM, El Tahlawy EM & Abd Allah HM 2011. Reproductive outcome, hormone levels and liver enzymes in agricultural female workers. *J. Adv. Res.*, 2(2): 185-189.

Jeng HA 2014. Exposure to endocrine disrupting chemicals and male reproductive health. *Frontiers in Public Health*, 2(55). www.frontierin.org

Kumar S 2004. Occupational exposure associated with reproductive dysfunction. *Journal of Occupational Health*, 46: 1-19.

Lawson CC, Schnorr TM, Daston GP, Grajewski B, Marcus M, McDiarmid M, Murono E, Perreault, D, Schrader SM & Shelby M 2003. An occupational reproductive research agenda for the third millennium. *Environmental Health Perspectives*, 111: 584–592.

Li D, Zhou Z, Qing D, He Y, Wu T, Miao M, Wang J, Weng X, Ferber JR, Herrinton LJ, Zhu Q, Gao E, Checkoway H & Yuan W 2010. Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. *Human Reproduction*, 25(2): 519–527.

Luderer U, Bushley A, Stover BD, Bremner WJ, Faustman EM, Takaro TK, Checkoway H & Brodkin CA 2004. Effects of occupational solvent exposure on reproductive hormone concentrations and fecundability in men. *Am. J. Ind. Med.*, 46(6): 614-26.

Mastrangelo G, Fedeli U, Fadda E, Milan G & Lange JH 2002. Epidemiologic evidence of cancer risk in textile industry workers: A review and update. *Toxicol Ind Health*, 18(4): 171-181.

Meeker JD, Rossano MG, Protas B, Padmanabhan V, Diamond MP, Puscheck E, Daly D, Paneth N & Wirth JJ 2010. Environmental exposure to metals and male reproductive hormones: Circulating testosterone is inversely associated with blood molybdenum. *Fertility Sterility*, 93(1): 130. doi:10.1016/j.fertnstert.2008.09.044.

Nagata C, Wada K, Tsuji M, Hayashi M, Takeda N & Yasuda K 2015. Association of hair dye use with circulating levels of sex hormones in premenopausal Japanese women. *The European J. Public Health*, 25(5): 895-9.

Padungtod C, Lasley B, Christiani DC, Ryan LM & Xu X 1998. Reproductive Hormone Profile among Pesticide Factory Workers. *J. Occupational and Envtal. Med.*, 40(12): 1038-1047.

Park HJ, Lee KM, Nam JK & Park NC 2005. A case of erectile dysfunction associated with chronic methyl bromide intoxication. *J. Impotence Res.*, 17: 207–208.

Pollack AZ, Schisterman EF, Goldman LR, Mumford SL, Albert PS, Jones RL & Wactawski-Wende J. 2011. Cadmium, lead, and mercury in relation to reproductive hormones and anovulation in premenopausal women. *Environmental Health Perspectives*, 119(8): 1156-1161.

Rachootin P & Olsen J 1983. The risk of infertility and delayed conception associated with exposures in the Danish workplace. *J. Occupational Med.*, 25(5): 394-402.

Sakr C J, Taiwo OA, Galusha DH, Slade MD, Fiellin MG, Bayer F, Savitz DA & Cullen MR 2010. Reproductive outcomes among male and female workers at an aluminum smelter. *J. Occupational and Envtal. Med.*, 52(2): 137-143.

Sánchez-García S, García-Peña C, Duque-López MX, Juárez-Cedillo T, Cortés-Núñez AR & Reyes-Beaman S 2007. Anthropometric measures and nutritional status in a healthy elderly population. *British Medical Council Public Health*, 7(2); doi:10.1186/1471-2458-7-2

Sani ZM, Abdullahi IL & Sani A 2018. Toxicity evaluation of selected dyes commonly used for clothing materials in urban Kano, Nigeria. *European J. Experi. Bio.*, 8(4): 26. doi:10.21767/2248-9215.100067

Sheiner EK, Shiner E, Hammel RD, Potashnik G & Carel R 2003. Effect of occupational exposure on male fertility: Literature review. *Industrial Health*, 41: 55-62.

Slater S & Oliver RTD 2000. Testosterone: Its role in development of prostate cancer and potential risk from use as hormone replacement therapy. *Drugs & Aging*, 17(6): 431-439.

Snijder CA, te Velde E, Roeleveld N & Burdorf A 2012. Occupational exposure to chemical substances and time to pregnancy: A systemic review. *Human Reproduction Update*, 18(3): 284-300.

Snyder PJ, Bhasin S, Cunningham GR, Matsumoto AM, Stephens-Shields AJ & Cauley JA 2016. Effects of testosterone treatment in older men. *The New England J. Med.*, 374: 611-624.

Soyinka OO 2017. Reproductive Characteristics of Male and Female Vat Textile-Dye Workers at Itoku, Abeokuta, Southwest, Nigeria. PhD Thesis, *Olabisi Onabanjo University, Ago Iwoye, Southwest, Nigeria*, 148p.

Tuzen M, Onal A & Soylak M 2008. Determination of trace heavy metals in some textile products produced in turkey. *Bull. Chem. Soc. Ethiopia*, 22(3): 379-384.

Xu X, Cho SI, Sammel M, You L, Cui S, Huang Y, Ma G, Padungtod C, Pothier L, Niu T, Christiani D, Smith T, Ryan L & Wang L 1998. Association of petrochemical exposure with spontaneous abortion. *Occupational and Environmental Medicine*, 55: 31-36.

Yucra S, Rubio J, Gasco M, Gonzales C, Steenland K & Gonzales GF 2006. Semen quality and reproductive sex hormone levels in peruvian pesticide sprayers. *Int. J. Occupational and Envt. Health*, 12(4): 355 – 61.