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AGE AT MENARCHE AND MENSTRUAL PATTERN OF VAT TEXTILE DYE-EXPOSED WORKERS AT ITOKU, ABEOKUTA, SOUTHWEST, NIGERIA

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ABSTRACT

Some changes in menstrual cycle characteristics have been associated with certain chemicals and/or occupational exposures. This study was conducted to investigate the likely effects of vat textile dyes on age at menarche and menstrual pattern of female subjects who are occupationally exposed to vat textile dyes. It involved a cohort of 39 female dye workers (aged, 16-49 years; minimum duration of exposure of two years) and 58 female unexposed subjects (aged, 17-48 years). Using a semi structured questionnaire, the menstrual history, cycle length, number of days of bleeding and degree of menstrual flow were obtained. Differences between the exposed and the control were tested for statistical significance using t-test for continuous variables and Chi-Square for categorical variables at P- value ≤ 0.05 level of significance.The mean age at menarche of the exposed (15.27±1.85 years) was not significantly different (p>0.05) from that of the control (12.83 ±4.50 years). The mean cycle length and number of days of bleeding were comparable to those of the unexposed (p>0.05). Among the exposed, 15.4% had irregular menstrual cycle which was not significantly different (p>0.05)from that of the control (10.9%). No premenstrual symptoms were recorded among the exposed population.It appeared that occupational exposure to vat dyes had no significant effects on age at menache, cycle length, number of days of bleeding, degree of menstrual flow and regularity of menstrual cycle of vat dye workers in Abeokuta.

Key words: Textile-dye workers, Menstrual Cycle, Age at Menarche, Pre- menstrual syndrome, Occupational Exposure.

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INTRODUCTION

"All chemicals are poisons, there is none that is not a poison but the dose makes the difference" Paracelcius (1493-1541). It is therefore important to test every chemical produced or in use for adverse effects. From epidemiological studies, documented effects of chemicals include reproductive disorders such as alterations in age at menache, menstrual pattern and hormone disbalance (Reutman *et al.,* 2002, Farr *et al.,* 2004,Park *et al.,* 2010, Hassani *et al.,* 2014).

Occupational exposure to chemicals and or occupational settings provides opportunities for testing of adverse effects of chemical exposure. This is because it involves a large quantum of exposures. This can be studied and then extrapolated to the public (Gardiner, *et al.,* 1982; Schrag and Dixon, 1985).

Dyes are coloured organic complex chemical compounds which have the ability of imparting colour on other substances; they have a long history and constitute an important component in our daily lives, they are either natural or synthetic (Bafana *et al.,* 2011; Kant, 2012; Ashfaq and Khatoon, 2014). Diverse categories of dyes include vat dyes, azo dyes, sulphur dyes, disperse dyes (Liaqat, 2009).

Human exposure to dyes occurs during manufacture, transportation, sale or application of dyes (Gardiner *et al.,* 1982; Rachootin, *et al.,* 1983, Liaqat, *et al.,* 2009). Dye powders can produce fine dusts, and solution can produce fine sprays of minute droplets that may be inhaled. During transfer of dyes from one container to another or scooping of dye, dye dust may gain access into the air and thus may be inhaled or settle on the human bodies (Liaqat, *et al.,* 2009).

Textile dyeing is an age-old process aimed at producing better quality and more fascinating textile products (Klemola, 2008). It forms an integral part of textile industries (Singhi *et al.,* 2005) where a wide range of chemicals are used for dyeing operation (Liaqat, 2009). Textile dyeing is indigenous to the people of Abeokuta, Nigeria. The name Abeokuta is traditionally inseparable from the a*dire* (meaning tie and dye) art tradition (Areo and Kalilu, 2013). The class of dyes used in Abeokuta is Vat dyes (Soyinka et al 2007a, b)

Several effects of dyes have been documented in literature ranging from organ effects to dermatitis, genotoxicity, mutagenicity, enzyme, immune, reproductive effects and oxidative stress (Rachootin and Olsen, 1983;Khanna and Sasseville, 2001; **Fanlo,*et al.*,2004;Singhi,*et al.*,2005;** Donya, *et al.,* 2012;Sargunar,*et al.,*2012; Al- Marshhedy, 2013; Güngördü, *et al.,* 2013;**Fernandes,*et al.*,2015**)

Specific effects of vat dyes include enzyme induction and subclinical effects on liver and kidney functions (Jascot and Costa, 1994; **Singh*et al.,* 2003;** Soyinka *et al.,* 2007a, b) There is however paucity of data on effects of occupational exposure to dyes (particularly vat dyes) on age at menarche and menstrual cycle characteristics such as length, duration, regularity and bleeding patterns (Hahn *et al.,* 2013).

Women with menstrual cycle disorders may have a higher risk of infertility (Harn *et al.,* 2013) among other adverse conditions.Altered age at menarche is not without their challenges, for example early menarche has been associated with breast cancer risk and many cardiovascular disease risk factors and metabolic syndrome (Lancet Oncol., 2012, Feng *et al.,* 2008).On the other hand, late menarche is associated with osteoporosis (Karapanou and Papadimitriou, 2010). However, factors affecting age at menarche include environmental influences and occupation (Heidi, 1986; Karapanou and Papadimitriou, 2010). Subclinical studies such as this are important, since lifestyle change and occupational control offer a possibility of correcting the adverse outcome. In this study, we examined the effects of occupational exposure to vat textile dye on menstrual cycle characteristics and age at onset of menarche.

MATERIALS AND METHODS

Study Design

This cohort study was based on interviews from 39 female subjects occupationally exposed to vat textile dye (exposed group) at Itoku, Abeokuta, Southwest, Nigeria and 52 non-exposed female subjects within Abeokuta environ. The requirements to participate in the study were to have had occupational exposure to vat textile dyes and to have worked with these dyes in the occupational setting and within the occupational environment for at least 2 years. For both the test and the control subjects, the females were non-pregnant and they were within reproductive age (between 16 and 49years; Morse *et al.,*2007), and had not, in the past three (3) months breastfed. For the control, those residing near dye workers (*adire* makers) were excluded. Also excluded were subjects with agricultural/pesticide related job and subjects involved in the manufacture or use of other chemicals such as those in lead (Pb) related occupation. The study was approved by the Scientific and Ethical Review Committee of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State. Consent was given by all the subjects that participated in this study.

Study Area

Itoku can be described as the ancestral home of *adire.*It is in the centre of Abeokuta and located few kilometers away from the popular “Olumo rock”. Itoku community is synonymous to dye work, sales of dyes and *adire* which is otherwise known as “kampala”. Abeokuta is the capital city of Ogun state, one of the thirty-six states of Nigeria, It lies between latitude 70 10 $^{/}$ N and 70 15 $^{/}$ N and longitudes 30 17$^{/}$ E and 30 26$^{/}$ E. It is a moderately large city, located about 100km northwest of Lagos; it covers an approximate area of about 40.63 km^{2}. (Ufoegbune et al., 2008)

Recruitment of the study population

Female textile dyers currently using vat dyes at

Itoku Abeokuta were identified at their various sheds and were informed of the aim and objectives of the study. Those who gave consent and fulfilled the inclusion criteria were recruited, they were altogether 39 subjects. Fifty-eight (58) non-exposed subjects were recruited as controls from among a group of schools and hospital administrative staff and attendants within Abeokuta environ. Neither the exposed nor the control reported the use of any other chemicals either occupationally or otherwise.

Sampling Technique and Sample Size

The sampling technique used in this study was purposeful and the total population of female vat dye workers was considered because it was manageable enough.

Data collection

Data collection method was by interview with the aid of a questionnaire and the use of a menstrual diary to capture the last menstrual period (LMP) of the subjects.

Interview

Interview was conducted using Interviewer-administered semi-structured questionnaires to gather information on reproductive health characteristics. The Interviewers were indigenous people trained to handle the questionnaire in either Yoruba (the local language) or English language.

Menacheal age: Age at menarche was defined as the age at which the subject first experienced menstruation (period) and it was captured in years. Menarche was described with reference to exposure. Those who had exposure after experiencing menarche were said to have post menacheal exposure while those who had exposure before experiencing menarche were described as having pre menacheal exposure. Age at menarche was assessed by recall method (Karapanou and Papadimitriou, 2010). The women responded to the question:” How old were you when you first saw your menses or period”. Double controls were used to derive effect of textile dye exposure on age at menarche. These were the post-manarcheally exposed vat textile dye workers and the original control subjects (unexposed subjects).The former fit as control because they had already experienced menache before exposure. With respect to the menarcheal aspect of the study, the pre-menarcheally exposed subjects were the true exposed subjects.

Menstrual cycle characteristics:All questions regarding the cycle characteristics were referred to the past three months before interview. It was observed that the participants did not have the proper understanding of cycle length (CL) and regularity of cycle; this led to the printing of a small calendar which was used as menstrual diary forobtaining the first day of the menstrual cycle or the last menstrual period (LMP). This enabled the calculation of the CL, the mean cycle length (MCL) and the regularity of menstrual cycle of each participant. The calendar contained the phone number of the principal investigator. This was kept by the participants and regularly checked by the principal investigator to capture LMP. Every participant was therefore encouraged to mark the first day of menses whenever it occurred, and this was done for four months consecutively, though some defaulted. The CL was defined as the number of days between the first day of menses in a particular month and the first day of the next menses in either that same month or another month (both days inclusive) and this was calculated from the calendar recordings. For each subject three such cycle lengths (CLs) were used to get the (MCL). Extreme or abnormal CL were either short (CL is < 24days)or long (CL is > 35 days) cycle length. Normal CL is between 24 and 35 days.

The variability of CL is the difference between the MCL and a particular month's cycle length. The regularity of the cycle was determined from the variability of each cycle from the mean. In any case, where the difference was more than or equal to seven, the cycle was adjudged to be irregular and regular if it is less than seven (Hahn et al 2013, Hsieh et al 2005). The monthly flow is said to be heavy if it comes with clots of blood and or there is need to change the sanitary towel (or improvised clothing material used by some of them) four or more times in the day. It is said to be light if it is possible to live the sanitary towel unchanged throughout the day, (for the reason of it not being soaked with blood) though this was not advocated. Medium flow involves the need for a change of sanitary towel between two or three times a day and presenting without clot.

Premenstrual or menstrual syndrome: A subject is said to experience premenstrual or menstrual syndrome if she has up to or more than four of the highlighted symptoms for each of the categories as presented in the questionnaire (Premenstrual Physical symptoms: abdominal bloating, abdominal discomfort or pain, breast tenderness or pain, weight gain, oedema, headaches, backaches, and/or nausea; Menstrual Symptoms: Cramps, Hot Flashes, Nausea, Fever, Diarrhoea, Sweats, Chill, Constipation, Headaches, Rectal Pain, Fainting, Dizziness).

Data analyses:

Data obtained from the questionnaire were analyzed using **statistical Package for Social Sciences (SPSS), versions 16.0 and 20.0. Descriptive statistics such as frequencies, percentages, means and standard deviations were generated as appropriate.** Chi-Square test was used to test for significant association between categorical variables; student's t- test was used to test the difference between the means of the two groups of continuous variables. Results were expressed as means ± SD. **P values ≤ 0.05 were considered significant.**

RESULTS

Table 1 shows the mean agesat menache of the textile-dye exposed and the control subjects. Among all the exposed population, only 11 had pre-menacheal exposure. Using the total female population of the unexposed group (n=58) and only 11 age-matched control, we observed similar (P>0.05) ages at menache among the groups. A further comparison was made while adding the post menacheally exposed subjects as a second set of controls.The mean age at menarche was higher in the pre-menacheally exposed subjects when compared with either the post-menarcheally exposed subjects or the original exposed group, but

Table 1: *Mean age at menarche in the pre-menarcheal exposed dye workers and control subjects*

	Mean ± SD (n)		t-test	P-value
	Pre-menarcheally Exposed subjects	Control		
Pre-menacheally exposed vs Total Control	15.27± 1.85 (11)	[a]12.83 ± 4.50 (58)	1.77	0.15
Pre-menarchially vs Age Matched Control	15.27± 1.85 (11)	[b]12.64 ± 4.27 (11)	1.88	0.22
	Pre-menarcheally exposed	Post-menarcheally Exposed		
Pre-menarcheally vs Post-menarcheal Exposed	15.27± 1.85 (11)	[c]14.55 ± 3.56 (22)	0.63	0.33

n= number of subjects P=probability; t= t-test, vs = versus, a=mean age at menarche when using the total number of control; b =mean age at menarche when using only age matched control, c= mean age at menarche when using the post-menarcheally exposed subjects as control subjects.

Table 2 presents the data on menstrual characteristics of the exposed and the unexposed populations. The entire population (100%) of women who responded to the question on cycle length had their cycle length within the normal range (23 -35days). The mean menstrual cycle lengths of the two groups were similar (P>0.05). The exposed subjects having irregular menstrual cycle were 15.4%, while 10.9% experienced such among the control. The proportions having regular to irregular menstruation were not significantly different (P>0.05) among the two groups. The proportion having less than 3 days or more than 6 days of blood flow (abnormal blood flow) to those having between 3 and 6 days (normal blood flow) in the exposed were similar (P>0.05) to that of the control. Also, the mean number of days of bleeding was similar (P>0.05) in the two groups. The proportions having heavy, medium and light menstrual flow in the exposed group was not significantly different (P>0.05) from the unexposed group.

Table 2 Menstrual Characteristics of the Textile - Dye Exposed and the Unexposed Female Population.

	Exposed	**Unexposed**	**t-test/X^2**	**P-Value**
	Frequency (%)		**X^2**	
Length of MC (days)				
<24	0(0.0)	0(0.0)	-	-
>35	0(0.0)	0(0.0)		
24-35	29(100)[a]	49(100)[a]		
NR	10	9		
Regularity of MC				
Regular	22(84.6)	41(89.1)	0.31	0.71
Irregular	4(15.4)	5(10.9)		
NR	13	12		
Duration of Bleeding (days)				
3-6 (normal)	37(97.4)	56(100)[a]	0.22	0.40
<3and>6 (abnormal)	1(2.6)	0(0.00)		
NR	1	2		
Description of Blood Flow				
Heavy	8(20.5)	12(20.7)	0.02	0.99
Medium	28(71.8)	42(72.4)		
Light	3(7.7)	4(6.9)		
	Mean ±SD		**t-test**	**P-Value**
Mean MCL (days)	29(27.79±2.72)	49(28.9±3.07)	1.6	0.11
Mean no. of days of bleeding	3.46±1.71	3.63±1.27	0.46	0.65

X^2 =chi square, t= t –test, %= percentage, MC= Menstrual Cycle, MCL = Menstrual Cycle Length, SD= standard deviation, P= probability, a= percentage only among the respondents (responded were not included)

Table 3 shows that none of the exposed population was seen to manifest either premenstrual or menstrual symptoms. However, a very small percentage (ranging from 1.7 to 7.0%) of the unexposed group showed premenstrual and menstrual syndrome.

Table 3: Pre-menstrual and menstrual syndrome among vat textile-dye exposed subjects and the un-exposed control

	Exposed	Unexposed	X^2	P-Value
Premenstrual Syndrome (Mental)				
[a]0-3	35(100)[y]	55(98.2)	-*	-*
=4	0(0.0)	1(1.8)		
NR	4	2		
Premenstrual Syndrome (Physical)				
0-3	35(100)	53(93.9)	-	-
=4[b]	0(0.0)	4 (7.0)		
NR	4	1		
Menstrual Syndrome				
0-3	31(100)	57(98.3)	-	-
=4	0(0.0)	1(1.7)		
NR	8	0		

X^2 =chi square, P=probability, a and b represent the number of individual signs and/or symptoms experienced by the subjects, * no Chi square and P values, NR =non-respondent, y=percentage only among the respondents (non-responded were not included)

DISCUSSION

Age at menarche of Female Dye Workers

The mean age at menarche (15.27±1.85years) recorded among vat textile dyers in this study seemed to be high even when compared with other studies that involved chemical exposures (Özen, and Darcan, 2011; Chen*et al.,*2011). Similar to this study, Freiman (2011) did not observe any significant association with age at menarche and PCB exposure. The age at menarche here reported among the control (12.83 ± 4.50 years) is comparable with what were reported in various part of the country by other authors (Uche and Okorafor,1979; Wright, 1990; Goon *et al.,* 2010; Amu and Bamidele, 2014)

Menstrual Characteristics of Female Dye Workers

At the present exposure level among vat textile dye exposed subjects at Itoku, Abeokuta, Nigeria, there were no effects on menstrual characteristics (such as mean cycle length, mean number of days of bleeding, flow pattern, and regularity). Both the exposed and the unexposed had their cycle length within the normal range (24-35) days. None was recorded to have abnormal cycle length. Lin *et al.,* (2013) in their study of exposure to solvents, reported shortened menstrual cycles in Liquid crystal display (LCD) manufacturing workers, the same with Cho *et al.,* (2001), in association with exposure to organic solvents. In contrast, Farr *et al.,* (2004) reported increased cycle length in association with pesticide exposure.

The mean cycle length reported in this study for the

exposed subjects was 27.79±2.72 days, and was found to be similar to that of the control 28.9±3.07days. Unlike in this study, Hassani (2014) reported a higher cycle length among the exposed compared with the control in the study of organic mixed solvent. Ajah *et al.,* (2015) reported 27.8 ± 3.14 days among girls in South - East, Nigeria while Iliyasu *et al.,* (2012) observed that 92% of University Students in Kano had cycle length between 21 and 35 days similar to both groups in this study. The prevalence of irregular cycles among the dye workers was 15.4%; the value recorded among the control was just 10.90%. Furthermore,Ajah (2015) also reported only 6%. The prevalence of abnormal duration of bleeding among the dyers was 2.6%. None of the control was observed to suffer from abnormal duration of bleeding. The prevalence of abnormal bleeding among the dyers was altogether 28.2% and among the control was 27.6%. The mean number of days of bleeding among the dye workers was 3.46±1.71. In their own study, Ajah *et al.,* (2015) reported 4.8 ± 1.14 days of bleeding while Hassani (2014) reported that duration of bleeding, amount of flow and also prevalence of long cycles and irregular cycles were higher in exposed groups than the control. Also, Hsieh *et al.,*(2005) observed that cycle length was greater among female workers engaged in thin film and ion implantation jobs than among non-fabrication workers and there was higher risk of short menstrual cycles among supervising engineers and photolithography workers than with non fabrication workers. On the other hand, in this present study, no significant difference was observed between the exposed workers who handled2-ethoxyethylacetate in the liquid crystal display manufacturing industry and referent groups (Hsieh *et al.,* 2005). Comparable to the study of Blatter et al (1993) among hairdressers with chemical exposure, we did not record any significant association with PMS and occupational exposure to textile dyes. Contrarily, Yao et al (2009) reported that shift work could increase the risk of PMS in female knitting workers.

CONCLUSION

Age at menarche and menstrual cycle characteristic such as cycle length, number of days of bleeding and degree of menstrual flow were not significantly affected by occupational exposure to textile dyes in Itoku, Abeokuta, Southwest, Nigeria. This result is limited by small sample size. It is hereby recommended that this be replicated using a multicentre approach so as to increase the sample size.

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COMPARATIVE RESPONSE OF BROILERS TO DIETARY INCLUSION OF ANTIBIOTICS, PROBIOTIC, ORGANIC ACID AND AN HERBAL PRODUCT

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ABSTRACT

An eight-week study was carried out to compare the effect of dietary antibiotics, probiotic, organic acid and an herbal product on live performance, haematology and serum biochemical constituents of broilers. One hundred and five broilers aged one week were assigned into seven treatment groups(control, organic acid, probiotic, neomycin, herbal product, oxytetracycline and colistin). Each group was replicated three times with five broilers per repliocate.The broilers were fed with diet containing Basal diet (T_1, Control), 1g/kg Hamecosal inclusion diet (T_2), 0.5g/kg Bio Vet-YC inclusion diet (T_3), 10mg/kg Neomycin inclusion diet (T_4), 0.5g/kg Livfit Vet inclusion diet (T_5), 10mg/kg Oxytetracycline inclusion diet (T_6) and 10mg/kg Colistin inclusion diet (T_7). The experimental diets and drinking water were supplied add libitum to the broilers for a period of eight weeks. Weight measurement were weekly and weight gain, feed conversion ratio, percentage digestibility were calculated. Haematological and serological analyses were carried out with standard methods. Results showed no significant difference ($P>0.05$) in weight gain of broilers in the different treatment groups with the highest gain recorded in birds on probiotics. With the exception of birds on antibiotic Colistin, feed intake of supplemented birds was lower ($P<0.05$) than the control group. Feed conversion ratio was better ($P<0.05$) in birds that received supplemental organic acid, probiotics, antibiotic neomycin and herbal product than the control group. Nutrient retention percentages varied non-significantly ($P>0.05$) in all treatments except ash and nitrogen free extract digestibilities ($P<0.05$). Among the haematological parameters, only Packed Cell Volume (PCV) was significantly affected by treatment effects ($P<0.05$). Similarly, only total protein differed significantly ($P<0.05$) among serum biochemical measurements.Fromthe findings of this study; probiotics, organic acids and herbal products can replace antibiotic growth promoters in broiler production.

Key Words:Broilers,feed additives, haematology,performance, serology.

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INTRODUCTION

The dietary inclusion of antibiotic growth promoters (AGPs) such as tetracycline, neomycin and colistin in poultry production for growth and maximization of genetic potential of poultry birds had witnessed an increase over the past few decades due to intensification of production. Increased weight gain in poultry birds has been reported as a result of continuous administration of low dose of antibiotics (Graham *et al.*, 2007). In a similar trend, antibiotics such as colistin, bacitracin and enramycin may change the composition of the gut flora in poultry birds to favour their growth as suggested in Ohya and Sato (1983). Also, it has been reported that some antibiotics may stimulate rapid metabolic processes in poultry birds, enhance feed consumption and consequently promote their growth (Engberg *et al.*, 2000; NOAH, 2004; Klotin, 2005; Miles *et al.,* 2006).

Consumers' apathy about the development of resistant strains of bacteria and its transfer to the human population, the presence of antibiotics residue in meat and meat products meant for human consumption has brought about the search for alternatives to antibiotics growth promoters