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Full Length Research Paper

Mixed chemical-induced oxidative stress in occupational exposure in Nigerians

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Exposure to single chemicals and associated disorders in occupational environments has received significant attention. Understanding these events holds great promise for risk identification, assessment and chemical induced disease prevention. Fifty (50) fasting male workers, age range 18-50 years exposed to chemical mixtures in a works department, mean duration 17.7±10.1 years and 30 controls matched for age, diet, sex and other demographic characteristics except exposure to chemicals were selected. Body mass index (BMI), antioxidant status and other biochemical indices including plasma proteins (total protein) and subsets, albumin and total globulins were determined in plasma. The BMI was similar between chemical workers (exposed) and controls ($p>0.05$). Uric acid level was significantly higher in the exposed than in the controls ($p<0.01$) probably in part up regulation to combat oxidative stress. Uric acid was also significantly positively correlated with BMI ($r=0.46$, $p<0.01$), probably to match the body chemical burden. Ascorbate was in contrast significantly lower in chemical workers than in controls ($p<0.001$), reduced by 91% level in controls. Border line inverse correlations between ascorbate, BMI and duration of exposure were evident. Copper (Cu) level, though slightly raised in chemical workers than in controls was not significant ($p>0.05$). Plasma proteins were significantly lower in chemical workers than in controls ($p<0.001$). Total globulins was significantly reduced in chemical workers ($p<0.01$). Other variables did not differ significantly. These data are consistent with the existence of oxidative stress in these chemical workers.

Key words: Chemical mixtures, occupational exposure, oxidative stress.

INTRODUCTION

The risk of chemical toxicity is recognized to be greatest in the rapidly industrializing and restructuring developing countries (WHO, 1992; Uzma et al., 2008). This is remarkably at variance with the developed countries with ample facilities to reduce over exposure and the toxic effects of chemicals. These risks are particularly enhanced in developing countries where there is often little information on the safe handling or transportation of chemicals in industry and agriculture. Accumulating evidence indicates that exposure to chemicals including the work place leads to generation of free radicals which if unaccompanied by available antioxidant leads to oxidative stress (Anetor and Adeniyi, 2001; Flora, 2005). Indeed oxidative stress is being increasingly recognized as a possible mechanism in the toxicity and carcinogenesis of various chemicals including heavy metals, organic and inorganic solvents, gases and acids encountered in the work place (Ramsey, 1992; Anetor, 1997).

Demonstration of excess free radical formation and deficit (absolute\relative) in antioxidant bioavailability in occupationally exposed individuals may serve as an early biochemical indicator of a pathophysiologic state. Owing to the high potential to damage vital biological systems,

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Table 1. Common chemicals in the work environment.

Heavy metals	Non metals	Organic solvents	Inorganic solvent	Gases
Lead	Cyanide	Chloroform	Mineral acids	Ammonia
Cadmium		Alcohol	H_2SO_4	Chlorine
Arsenic		Ether	HCl	phosgene
Mercury		Formalin	Alkalis	Freon
		Phenol		H_2S
		Xylene		NO_2
		Toluene		
		Benezidine		
		Petrol		
		Greases		

Source: Modified from Ramsey (1992).

Table 2. Occupation and chemicals to which workers are exposed.

Occupation	Benzene	Gasoline	Mineral acid	Ethylene	Toluene	Phenyl mercury	Lead	Cadmium
Automechanics	+	+	+	-	+	-	+	+
Panel beating	+	+	-	+	-	+	+	+
Battery charging	-	-	+	-	-	-	+	+
Welding	+	-	-	+	-	-	+	-
Painting	+	+	-	+	+	+	+	+
Auto/ electrical work	-	-	-	-	-	-	+	-

toxic oxygen species (TOS) have now been incriminated in aging and over a hundred diseases including cancer causation usually preceded by genome instability (Ames et al., 1993).

The main objective of this study was to investigate if prolonged exposure of workers, occupationally exposed to several chemicals induces oxidative stress and implications for genome instability. This has not been determined in Nigerians in particular and most developing countries in general, where knowledge of the harmful effects of chemicals is poorly recognized and micronutrient deficiency disorders (MDDs), the major basis of the antioxidant system and nutrigenomics are common.

In this study simple (common) antioxidants, such as vitamin C, uric acid, albumin, copper (active constituent of SOD) were determined in exposed and unexposed materials and methods.

MATERIALS AND METHODS

Chemicals in the work environment

The chemicals to which these workers are exposed have been described (Ramsey, 1992) and are shown in Table 1. Workers may be exposed to any of these in combination or in compounds of which these are constituents e.g. in welding, or in paints (Table 2).

Fifty (50) male chemical workers from various occupational groups as shown in Table 3 exposed to the chemicals listed in Table 1 were investigated. The distribution of the chemical workers

Table 3. Occupational groups of workers.

Occupation	Number
Auto mechanics	18
Battery workers	4
Home painters	11
Panel beaters	6
Auto electricians and vulcanizers	7
Welders	4

is also shown in Table 3. They were classified into two broad classes; those severely exposed and those mildly exposed. The mean duration of exposure of the chemical workers was 17.7 ± 10.8 years. The age range of the workers was 18-50 years.

Thirty (30) non-exposed male subjects matched for age (17-52 years) served as controls. Dietary intake as assessed by 24 h dietary recall especially as regards micronutrients was similar between exposed and unexposed subjects. The body mass indexes (BMI) of both chemical workers and controls were computed according to the standard formula:

$$\mathrm{BMI} = \frac{\mathrm{Weight}(\mathrm{kg})}{\mathrm{Height}(\mathrm{m}^2)}$$

About 10 ml of venous blood was collected from all study particip--ants according to standard procedure. Albumin was determined by dye binding method using bromocresol green while ascorbic acid was determined by a standard Spectrophotometric method. Fasting plasma glucose was assayed by the glucose oxidase method. The

Table 4. BMI, glucose and the antioxidants, ascorbic acid, copper and uric acid in chemical workers and controls.

Parameter	Chemical workers (n = 50)	Controls (n = 30)	t	P
BMI (kg/m^2)	22.32 ± 3.54	21.87 ± 2.19	0.71	>0.05
Glucose (mg/dl)	94.28 ± 22.62	88.37 ± 18.46	1.27	>0.05
Ascorbic acid (mg/dl)	0.22 ± 0.13	0.42 ± 0.21	4.57	<0.001
Copper (µg/dl)	68.64 ± 18.24	62.60 ± 17.40	1.47	>0.05
Uric acid (mg/dl)	5.83 ± 1.23	5.03 ± 1.33	2.67	<0.01

Table 5. Total protein, albumin, total globulin, total and ionized calcium in chemical workers and controls.

Parameter	Chemical workers (n = 50)	Controls (n = 30)	t	P
Total protein (g/dl)	6.91 ± 0.73	7.48 ± 0.58	3.81	<0.001
Albumin (g/dl)	4.08 ± 0.84	4.11 ± 0.53	0.19	>0.05
Total globulin (g/dl)	2.83 ± 0.11	3.37 ± 0.05	3.62	<0.01
Total calcium (mg/dl)	8.66 ± 1.38	8.97 ± 0.81	1.28	>0.05
Ionized calcium (mg/dl)	3.80 ± 0.60	3.80 ± 0.34	0.15	>0.05

antioxidant, uric acid was determined by standard Spectrophotometric method. The micronutrient, copper was determined with atomic absorption spectrophotometry (Buck Scientific, Germany). Total protein was evaluated by standard biuret reaction. Total globulin level was computed by subtracting albumin level from total protein concentration. Total calcium level was evaluated by a Spectrophotometric method while ionized calcium level was computed by a standard formula.

All analyses were subjected to quality checks to ensure reliability of generated data. Results were only accepted if QC data fell between mean values ± 2SD.

All data were expressed as mean ± SEM. Results were analyzed using the Students't-test and correlation studies using Pearson's Product Moment Correlation coefficient. Differences between values in chemical workers and controls were accepted as significant at 5% (P<0.05) level.

RESULTS

Body mass index was similar between chemical workers and controls (Table 4). The level of the endogenous and dynamic antioxidant uric acid was significantly higher in chemical workers than in controls (P<0.01). Additionally, uric acid level was positively correlated with BMI and duration of exposure to chemicals (r = 0.46 p<0.001). Ascorbic acid level in contrast to that of urate was significantly reduced in chemical workers than in controls (P<0.001). Indeed the level in exposed workers was nearly 50% in controls. In addition, ascorbic acid level was negatively correlated with BMI but this was not significant. It was also positively correlated with duration of exposure but also did not reach significant level. The antioxidant, copper was raised in chemical workers but did not reach significant level (P>0.05). Total protein and globulin levels were significantly lower in chemical workers than in controls (P<0.001 and P<0.01) respectively (Table 5). Albumin level was however not significantly different between exposed and unexposed populations. Total and ionized calcium like albumin, did not show any significant differences between exposed and unexposed subjects. No differences were observed according to intensity of exposure (that is, no dose dependence was demonstrated).

DISCUSSION

Many chemicals used in industries and to which over exposure may occur have been demonstrated to elicit a number of biochemical responses (Chow, 2002). The body in an attempt to adjust or accommodate the effect of chemicals responds in a variety of ways, one of which is antioxidant/oxidant imbalance (oxidative stress) (Halliwell, 1988; Halliwell and Guterridge, 1992; Cross et al., 2002).

This study demonstrates that these chemical workers have increased oxidative stress. The significantly reduced ascorbate level in exposed workers compared to unexposed subjects; most probably reflects increased demand for this antioxidant to counter the excessive free radical burden. Vitamin C has generalized antioxidant property and possesses preponderance in scavenging ability for the more common toxic oxygen species (TOS) (Buettner and Jurkiweicz, 1996). Thus, the demand for ascorbate unlike limited or specific antioxidants will be high.

This significant reduction in ascorbate level probably confirms the suggestion that vitamin C is the first line of defence (Frei et al., 1989) in the antioxidant defence system. The inverse correlation between BMI and ascorbate (Table 6) implies that the bigger the BMI the greater the chemical burden sequestered leading to high ROS and in turn the greater the demand for ascorbate and consequently a greater fall in its antioxidant activity.

Table 6. Correlation of BMI and antioxidants against duration of exposure to chemicals.

Parameter	R	P
BMI	0.32	<0.002
Uric acid	0.26	>0.05
Copper	0.00	>0.05
Ascorbic acid	0.04	>0.05

Table 7. Correlation of antioxidants, glucose and copper against BMI in chemical workers.

Parameter	R	P
Ascorbic acid	-0.11	>0.05
Glucose	0.16	>0.05
Copper	0.00	>0.05
Uric acid	0.46	<0.001

The deficit in this potent antioxidant may increase risk of cancer. Vitamin C protects against DNA damage (Cail et al., 2001; Diatrich et al., 2003). DNA mutation in the absence of appropriate repair mechanisms may be the beginning of initiation phase of the carcinogenic process (Byer et al., 1992).

Uric acid is a metabolic or endogenous antioxidant. The elevated urate level in these workers is an adaptive response or mechanism against the toxic effect of chemicals. This is consistent with earlier reports of elevation of serum uric acid concentration occurring as a physiologic response to oxidative stress providing counter regulatory increase in antioxidant defenses (Simic and Jovanic, 1989; Ames et al., 1981). Gittleman et al. (1994) have reported that uric acid may be a consistent and reliable biomarker of significant exposure to toxicants especially lead. Uric acid also acts as a repair agent of oxidative damage to DNA bases (Simic and Jovanic, 1989), probably another reason for the increase. This increase may also be an attempt to raise the level of the frontline antioxidant, ascorbate (Sevanian et al., 1991).

The positive correlation between urate and BMI ($r = 0.46$, $P < 0.01$, Table 7) implies that the greater the BMI, the greater the chemical burden and in turn the greater the adaptive response to curtail the oxidative stress including forestalling probability of genotoxic events. This appears consistent with the inverse response of ascorbic acid.

Of recent, owing to the far reaching consequences of genotoxicity, many investigators have focused on 8–Hydroxyl–2– deoxyguanosine (8–0HdG), a metabolite of DNA damage. 8–0HdG is a hydroxyl radical damaged guanosine nucleotide that has been excised from DNA by endonuclease repair enzymes (Floyd, 1990). There is an inverse relationship between uric acid and 8 – 0HdG (Yoshida et al., 2001). Increasing levels of uric acid are accompanied by a decrease in 8–0HdG levels, an evidence consistent with that from patients with acute ischemic strokes in whom a 12% increase in the odds of good clinical outcome for each milligram per deciliter increase of serum uric acid has been observed (Chamoro et al., 2002).

8–0HdG reflects an individual's mutation potential and therefore cancer risk (Kasai et al., 1986; Floyd, 1990). The increase in urate in these workers may be considered an adaptive response to protect the most important biological material in the human body, DNA; preventing genetic damage that may give rise to mutation and ultimately a neoplastic state. Importantly, it confirms the existence of oxidative stress previously suggested by the marked reduction in ascorbate level.

The antioxidant action of uric acid is partly manifested by interaction with ascorbic acid (Servanian et al., 1991) that is particularly evident in species that lack the ability to synthesize this vitamin. Urate not only behaves as a radical scavenger but also stabilizes ascorbate in biological fluids. The uric acid pool appears to expand to contend with the body burden of chemicals as an adaptive response. Increase in urate level has been inversely correlated with the marker of genotoxicity, 8–0HdG, and may suggest reduced risk of genetic damage; it is possible that when ascorbate level falls below a certain critical or threshold level in the presence of immune paresis as suggested by the marked reduction in total globulin level in our exposed subjects, urate protection may decline and may give way to genetic damage, mutation and the onset of carcinogenesis. This appears very consistent with the recent concept of the role of micronutrients on genome stability and carcinogenesis (Ames and Wakimoto, 2002; Fenech, 2003). Various micronutrients play an important role in DNA metabolism and DNA repair (Ames and Wakimoto, 2002; Fenech, 2003). Understanding the requirement of micronutrients needed to maintain genome stability is an essential step in the definition of optimal diets for the prevention of cancer and other diseases caused by genome damage, a current focus in disease prevention (Ames and Wakimoto, 2002; Fenech, 2003; Fenech, 2004; Fenech et al., 2005). This is very important in developing nations where protective micronutrients are deficient (Gibson, 1994; Underwood and Smitiasiri, 1999; Smith, 2000; Anetor and Agbedana, 2001).

Serum copper is most often raised as a component of ceruloplasmin, an acute phase reactant and antioxidant (Tolonen, 1990). Copper is also a component of the potent cytosolic antioxidant copper-zinc superoxide dismutase (Cu-Zn SOD). The non significant increase in Cu level may in part be a reflection of the depressed globulins; the major transporter of Cu is an alpha 2 - globulin, ceruloplasmin.

The decrease in total globulin probably reflects humoral immunosuppressive effect of the chemicals to which these workers are exposed. This is also in accord with

immune paresis in these workers (Anetor and Adeniyi, 1998). This taken with oxidative stress suggests the combined existence of both compromised antioxidant and immune systems defense in these workers.

An integral part of the signaling systems is calcium ion. The non significant reduction in calcium level may be a corroboration of the observed increase in urate level as an adaptive response to oxidative stress.

If the elevated uric acid was a consequence of renal damage, calcium level would have been significantly reduced due to renal osteodystrophy as the chemical assault on the kidney is a chronic event (Goyer and Ryne, 1973; Anetor, 2002). The effect of oxidative stress on a particular system was evaluated by looking at the pancreas, to assess the possible pancreatotoxic effect, fasting plasma glucose and its possible effect on glucose stimulated insulin released (GSIS) was higher in chemical workers, probably indicating a trend of chemical-induced chronic hyperglycemia.

Conclusion

Oxidative stress exists in these workers exposed to a mixture of chemicals in their occupations. Alteration in the humoral immune system and its implications in the evolution and pathogenesis of a number of non communicable diseases especially cancer is evident. Uric acid and ascorbic acid determinations may serve as simple tools of detecting oxidative stress from chemical exposure and risk of genome instability. Understanding the interaction of these intricate events is important in the prevention and amelioration of the unrecognized but existing chemical -induced oxidative stress resulting from chronic exposure to chemicals in the work place. This appears an occult problem in Nigeria and many other developing countries that must industrialize and restructure their economy. Counteracting oxidative stress appears a desirable activity to strike a balance and reduce the risk of genome instability.

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