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Research Article

Serum Levels of Selected Inflammatory Markers in Farm Workers Exposed to Organophosphate Pesticides

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ABSTRACT
Despite the acute and chronic adverse effects associated with the use of organophosphate pesticide (OPs), they continue to be used indiscriminately by farm workers. Exposure to various toxicants such as OPs has been shown to evoke various immune responses including inflammatory processes which have been implicated in the aetiology and pathogenesis of several pathological disorders. Although occupational exposure to OPs is linked with dysregulated immune responses, information on the group of farm workers at high risk of dysregulation is lacking. This study therefore, determined the differences in the serum levels ofselected inflammatory markers in farmers and pesticide applicators exposed to OPs. One hundred and eighty adult males comprising 60 pesticide applicators (PA), 60 farmers exposed to OPs for more than 5 years, and 60 apparently healthy adults without occupational exposure to OPs who served as controls were enrolled into this study. Differential leucocyte count was determined using thin film microscopy and Neutrophil-lymphocyte ratio (NLR) was calculated. Serum level of C-reactive protein (CRP) was determined using ELISA and activity of Nicotinamide adenine dinucleotide phosphate (NADPH)oxidase (Nox) was determined spectrophotometrically. The mean serum level of CRP and Nox activity were significantly higher in PA and farmers compared with the controls. In contrast, NLR was significantly lower in PA and farmers compared with the controls. However,the mean serum level of CRP was significantly higher in PA compared with farmers. Long term exposure to OPs is associated with systemic inflammation which is more pronounced in PA. Also, long term exposure to OPs seemed to be associated with heightened phagocytic activities which could promote oxidative injury of the lung.

Keywords: *Menopause, Obesity, Nutritional status, Women*

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INTRODUCTION

Previous studies showed that pesticides such as persistent organic pollutants (POPs) especially, organochlorine pesticides (OCPs) are relatively safe to use but persist in the environment and bioaccumulate in the food chain. Due to serious adverse effects associated with OCPs exposure, they were banned and replaced with pesticides such as organophosphate pesticides (OPs) which are acutely toxic but do not persist in the environment beyond a few months (Szmedra, 1994; Pesticide Action Network, 2017).

Dichlorvos, chlorpyrifos and dimethoate are OPs which are frequently sprayed in cocoa, cashew, orange and mango plantations of South-Western Nigeria, a tree crop belt of the country (Sosan *et al*., 2009). Of all, dichlorvos (DDVP), a class 1b highly hazardous OP according to International Standard Organization (ISO) with trade names Siege, Snipers, DDforce, Didwell is the commonest and the most widely used in this region. Although, the chemical structures of OPs vary considerably, they have a similar physiological mode of action.

The presence of non-selective toxic compounds in OPs which have been reported to be harmful to humans that are occupationally or environmentally exposed indicates the need for adequate regulation of the use of OPs (Anetor *et al*., 2001). Aside the widely reported neurotoxic effects of OPs (Anetor *et al*., 2001; Banks and Lein, 2012), we have also showed that OPs have adverse effects on the immune system of exposed farm workers (Yaqub *et al*., 2014a, 2014b, 2014c).

Inflammation is a protective response that involves the interaction of a number of cellular and molecular components. These interacting elements are coordinated and controlled in order to deliver appropriate response to irritation or infection that initiated the inflammation (Kaplan, 2017). Emerging evidences show that inflammation is of vital function in the pathophysiology of organophosphate pesticides (OPs) toxicity in humans and since it precedes immunosuppression, hypersensitivity and autoimmunity (Luster *et al*., 1992; Galloway and Handy, 2003), it must be accorded appropriate importance. Although inflammation plays a vital role in the pathophysiology of OPs toxicity (Medzhitov, 2008), the inflammatory state that accompanies OPs exposure does not completely fit into the classical definition of acute or chronic inflammation, in that it is not accompanied by infection and no massive tissue injury does occur. Additionally, the magnitude of activation of inflammatory response is not as pronounced. This indicates that OPs exposure has unique inflammatory features and its causes are far from being fully understood. However, it may be suggested that OPs act as toxicants and interact with immune cells to cause cellular/tissue damage and/or homeostatic imbalance of one or several physiological systems. This might be identified as the basis of the inflammatory process which progresses to a dysregulation implicated in hypersensitivity, immunosuppression or autoimmunity associated with occupational/environmental exposure to pesticides (Medzhitov, 2008; Luster *et al*., 1992; Galloway and Handy, 2003).

Similarly, excessive accumulation of OPs particles during cumulative exposure may trigger an imbalance between cellular and molecular components of inflammation. In addition, a molecular component of a pesticide residue may activate an inflammatory response via interaction with receptors on the cell surfacesthereby enhancing or inhibiting the physiological function of such cells, leading to allergy, autoimmunity, immunosuppression or increased cancer susceptibility and risk of infections (IPCS, 1996; Luster *et al*., 1992; Galloway and Handy, 2003). The mobilization of components of the innate immunity and specific adaptive immune response to clear the pesticide residue irritation are the final result of inflammation (Turner, 1994; Medzhitov, 2008; Sarwar, 2015).

Acute phase proteins such as C-reactive protein (CRP) have been well recognized for their application in diagnosis and prognosis of various human diseases (Kao *et al*., 2006). CRP is synthezised by the liver mainly as acute phase protein in response to tissue damage (Yudkin *et al*., 1999). CRP levels rise in serum or plasma within 24 to 48 hours following acute tissue damage, reach a peak during the acute stage and decrease with the resolution of inflammation or trauma (Schultz and Arnold, 1990). The increase inplasma CRP concentration in human may last for several days before decreasing to normal levels (Dixon, 1984). CRP can be used together with other inflammatory markers to evaluate an individual for acute or chronic inflammatory conditions (Pearson, 2003).

Reports have shown that serum concentrations of various acute-phase proteins including CRP are elevated in workers occupationally exposed to air pollutants (Ohlson *et al*., 2010, Saber *et al*., 2013). Similarly, CRP has been reported to be significantly elevated in factory workers exposed to pesticides (Khan *et al*., 2010). However, its diagnostic and prognostic performance in farm workers still needs further research.

Neutrophil to lymphocyte ratio (NLR) is calculated as a simple ratio between the absolute neutrophil count and the absolute lymphocyte count; two different parts of the total white blood count (Gary *et al*., 2013). Ohayo-Mitoko (1999) reported reduced neutrophil count and raised lymphocyte counts resulting in reduced NLR in exposed farm workers compared to controls. This report indicates altered cellular immune response in the farm workers. NLR has been reported to correlate with other inflammatory biomarkers and was proposed as predictive marker of morbidity and mortality in risk assessment of chronic disorders (Uthamalingam *et al*., 2011; Han *et al*., 2013). However, the usefulness of NLR to determine inflammation in farm workers exposed to OPs has not been previously reported.

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Nox) represent a class of hetero-oligomeric enzymes whose primary function is the generation of reactive oxygen species (ROS). In the vasculature, Nox-derived ROS contribute to the maintenance of vascular tone and regulate important processes such as cell growth, proliferation, differentiation, apoptosis, cytoskeletal organization, and cell migration. Under pathological conditions, excessive Nox-dependent ROS formation, which is generally associated with the up-regulation of different Nox subtypes, induces dysregulation of the redox control systems and promotes oxidative injury of the cells (Lambeth, 2007).

Although occupational exposure to OPs is linked with dysregulated immune responses, information on the group of farm workers at high risk of dysregulation is lacking. This study therefore, determined the differences in the serum levels ofselected inflammatory markers in farmers and pesticide applicators with long term exposure to OPs.

MATERIALS AND METHODS

Study participants

After obtaining an approval from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Committee and written informed consent from each participant, 120 adult male farm workers (47 ± 7 years) and 60 age and sex matched civil servants (46 ± 10 years) that neither use OPs nor practice farming who served as controls were enrolled into this study. The farmworkers were further divided into two groups consisting of 60 pesticide applicators (PA) and 60 farmers.

Venous blood (10 ml) was obtained from each study participant and 3 ml was dispensed into EDTA sample bottlesfor the determination of complete blood count. The remaining 7 ml was dispensed into plain sample bottles to obtain serum which was stored at -20ºC until analyzed. Differential leucocyte count was determined using thin film microscopy as described by Chessbrough (2000) and Neutrophil-Lymphocyte ratio (NLR) was calculated from the absolute values of neutrophils and lymphocytes. Serum level of C-reactive protein (CRP) was determined using ELISA as described by the manufacturer (Elabscience Biotechnology Company. Ltd. USA) and activity of NADPH oxidase (Nox) was determined spectrophotometrically as previously described by Li *et al*. (2002). Briefly, the NOx activity was evaluated based on a colorimetric assay that measures the reduction of cytochrome c by NADPH-Cytochrome c reductase in the presence of NADPH. The reduction of cytochrome c results in the formation of distinct bands in the absorption spectrum and the increase in absorbance at 550 nm is measured with time.

Data analyses were done using ANOVA, Student's t-test and Pearson correlation coefficient. *P*-values less than 0.05 were considered as statistically significant. The results are expressed as Mean ± Standard Error of Mean (SEM).

RESULTS

As shown in Table 1, the mean serum level of CRP, and serum activity of Nox were significantly higher in farm workers compared with the controls. In contrast, NLR was significantly lower in farm workers compared with the controls.

Table 1:
Serum levels of selected inflammatory markers in farm workers exposed OPs and controls

	Controls (n = 60)	Farmworkers (PA+ F) (n = 120)	*P*-value
Nox(U/mL)	4.8 ± 0.5	7.9 ± 0.5	<0.001*
NLR	1.4 ± 0.0	0.8 ± 0.1	<0.001*
CRP (mg/L)	7.2 ± 0.8	10.1 ± 0.8	<0.001*

**Significantly different from controls at P<0.05, Nox = NADPH oxidase, NLR = Neutrophil-Lymphocyte Ratio, CRP = C-reactive protein, PA = Pesticide applicator, F = Farmer*

Similarly, the serum level of CRP and activity of Nox were significantly higher in PA compared with the controls and in farmers compared with the controls. NLR was significantly lower in PAcompared with the controls and in farmers compared with the controls (Table 2). Comparing PA with the farmers, it was observed that the mean level of CRP was higher in PA compared with the farmers (Table 3).

Table 2:
Serum levels of selected inflammatory markers in Pesticide Applicators, farmers and controls

	Controls (n = 60)	Pesticide Applicators (n = 60)	Farmers (n = 60)	*P*-value[a]	*P*-value[b]
Nox (U/mL)	4.8 ± 0.5	7.3 ± 0.7	8.6 ± 0.6	0.019*	<0.001*
NLR	1.4 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	<0.001*	<0.001*
CRP (mg/L)	7.2 ± 0.8	12.6 ± 1.4	7.6 ± 0.7	<0.001*	0.041*

*Significant at *P*<0.05, [a]Pesticide applicators vs controls, [b]farmersvs controls, Nox = NADPH oxidase, NLR = Neutrophil-Lymphocyte Ratio, CRP = C-reactive protein

Table 3:
Serum levels of selected inflammatory markers in pesticide applicators and farmers

	Pesticide Applicators (n = 60)	Farmers (n = 60)	*P*-value
Nox(U/mL)	7.3 ± 0.7	8.6 ± 0.6	0.054
NLR	0.8 ± 0.0	0.8 ± 0.0	0.721
CRP (mg/L)	12.6 ± 1.4	7.6 ± 0.7	0.014*

*Significant from farmers at *P*< 0.05, Nox = NADPH oxidase, NLR = Neutrophil-Lymphocyte Ratio, CRP = C-reactive protein

DISCUSSION

Long term exposure to OPs has been associated with a few adverse effects on the immune system (Yaqub *et al*., 2014a, 2014b, 2014c). NOX activity is responsible for the killing of intracellular pathogens. In oxygen-dependent myeloperoxidase-independent mechanism, activated Nox uses oxygen to oxidize the NADPH resulting in the production of superoxide anion (Lambeth, 2007; Manea, 2010). In this study, the serum activity of Nox was significantly higher in all the farm workers compared with the controls. This observation is consistent with previous experimental studies (Binukumar *et al*., 2010; Owoeye *et al*., 2012). We have also previously reported several folds increase in ROS generation in farm workers exposed to OPs (Yaqub *et al*., 2014b). Observation from this study might indicate heightenedphagocytic/respiratory burst activities by phagocytes with a view to eliminating ingested OP molecules. This could however result in oxidative lung injury occasioned by the excessive release of ROS.

White blood cells (WBCs) are responsible for protection against exogenous and endogenous toxic substances (Han *et al*., 2013). Previous reports have shown that the ratio of some immune cells such as the Neutrophil-Lymphocyte ratio (NLR) are better cellular inflammatory markers than the absolute counts of the cells. NLR was reported to correlate with other inflammatory biomarkers and was proposed as a predictive marker of chronic inflammation in risk assessment of chronic disorders (Uthamalingam *et al*., 2011).The observed significantly lower NLR in the farm workers (PA and farmers) compared with the controls is in line with the reports of Desi *et al*. (1992) and Yaqub *et al*. (2014a) which showed lower counts of neutrophils but raised counts of lymphocytes and eosinophils in PA and farmers exposed to OPs compared with the controls. This observation might indicate that there is a shift in immune response towards the lymphocytes andcould provide basis for strong association between chronic exposure to OPs and chronic inflammation. Considering migration of immune cells, neutrophils are early migrators, followed by macrophages/monocytes and later lymphocytes. Thus, when lymphocytes are increased, it indicates an on-going chronic inflammation. Our observation might suggest that NLR could be a simple marker of inflammation and cellular immune responses dysfunction in individuals with long term exposure to OPs.

Acute inflammation is associated with the production of pro-inflammatory cytokines. These cytokines stimulate the liver to produce acute phase proteins including CRP among others (Cals *et al*., 2010). In clinical terms, measurement of acute phase proteins is useful to assess the degree of inflammation in an individual and also to assess the response to therapy (Cals *et al*., 2010). C-reactive protein binds to antigens, activates the complement pathways which facilitate the clearance of the antigen either by complement-mediated lysis or increased phagocytosis. Its serum concentration increases in thousand folds during an acute phase response (Pepys and Baltz, 1983; Gabay and Kushner, 1999; Cals *et al*., 2010). The observed significant elevation in CRP levels in all the farm workers compared with the controls indicates heightened inflammation in individuals with long term exposure to OPs. Inhalation,which is the major route of exposure of farm workers to OPs may cause irritation of the alveoli characterized by localized inflammatory response, localized vasodilatation of blood vessels and attraction of phagocytes especially, neutrophils and macrophages (Keifer *et al*., 1996; Costa, 2006; Uthamalingam *et al*., 2011). This might have led to systemic inflammatory response where cytokines such as interleukin-6 (IL-6) would have acted on the liver to increase the production of CRP. In addition, the liver is the main organ involved in the metabolism of OPs hence; it is exposed to both intact pesticide residues that entered through the lungs and products of hepatic biotransformation of OPs (Costa, 2018). Heightened inflammation with persistent CRP synthesis by the liver could be involved in the pathogenesis of OPs-associated liver damage. Owoeye *et al*. (2012) reported histological liver damages in experimental animals exposed to OPs. Similarly, Yaqub *et al*. (2014c) reported altered liver function in farm workers exposed to OPs.

Although farm workers are all exposed to the immune-toxic effects of OPs, our study observed that the effects could be more pronounced in PA compared with the farmers as the CRP level in PA was significantly higher than the farmers. This observation might not be surprising as the PA are exposed to higher dose of OPs (as many of them do not use protective wears consistently) than the farmers who usually work on the farm after OPs application has been concluded by the farmers.

It could be concluded from this study that long term exposure to OPs is associated with systemic inflammation which is more pronounced in PA. Also, long term exposure to OPs seemed to be associated with heightened phagocytic activities which could promote oxidative injury of the lung. Therefore, farm workers exposed to organophosphate pesticide would benefit from consistent and correct use of protective personal equipment as well as routine clinical examinations to facilitate prompt diagnosis of lung and liver damages

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