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Haematological and Immunological Indices in Nigerian Farmworkers Occupationally Exposed to Organophosphate Pesticides

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ABSTRACT

Experimental studies have shown numerous health disorders associated with occupational exposure to organophosphate pesticides but evidence of impaired immune functions by pesticides in humans is scarce. This study determined complete blood count (CBC), serum immunoglobulin classes (IgE, IgA, IgM, IgG),acetylcholinesterase (AChE) activity and skin sensitivity prick test to common environmental allergens in 60 farm workers (30 pesticide applicators and 30 farmers) exposed to organophosphate (OP) pesticides compared with30 apparently healthy, non-exposed workers. Serum AChE activity, mean PCV level, total white blood cell count (TWBC) and neutrophil count were significantly reduced while the mean lymphocyte count and eosinophil count were significantly raised in both pesticides applicators (PA) and farmers compared with controls. However, mean monocyte count was significantly raised in PA compared with controls. Also, serum activityof AChE and mean serum IgM level were significantly reduced while the mean monocyte count was significantly raised in PA compared with farmers. This study shows that pesticides applicators are more exposed to effects of organophosphate than farmers.

***Key words**: Farm workers, immunosuppression, organophosphate exposure, pesticide applicators*

Mesleki Organfosfat Pestisitlere Maruz Kalmış Nijeryalı Çiftçilerin Hematolojik ve İmmünolojik Endeksleri

ÖZET

Deneysel çalışmalar organfosfat pestisitlere mesleki maruziyet ile ilişkili çok sayıda sağlık bozuklukları göstermiştir, ancak insanlarda pestisitler tarafından bozulmuş immün fonksiyon bulguları azdır. Bu çalışma, pestisitlere maruz kalmış 60 tarım işçisi (30 pestisit uygulayan ve 30 çiftçi) ve karşılaştırma yapmak üzere görünüşde sağlıklı, organikfosforlara maruz kalmamış 30 işcide, tam kan sayımı (CBC), serum immunglobulinleri (IgE, IgA, IgM, IgG), asetilkolinesteraz (AChE) aktivitesive ve ortak çevresel alerjenlere deri prick testi duyarlılığı yapılmıştır. Kontrol grubu ile karşılaştırıldığında hem pestisitler uygulamacılar (PA) ve çiftçilerin serum AChE aktivitesi, ortalama lenfosit sayısı ve eozinofil sayısı önemli ölçüde yükselmişken, PCV düzeyi, toplam beyaz kan hücresi sayımı (WBC) ve nötrofil sayısı önemli ölçüde azalmıştı. Ancak, monosit sayısı kontrollerle karşılaştırıldığında PA'da önemli ölçüde armıştı Ayrıca, serum AChE aktivitesi ve serum IgM seviyesi çiftçiler ile karşılaştırıldığında PA'da anlamlı şekilde azalmışken, monosit sayısı anlamlı oranda artmıştı. Bu çalışma pestisit uygulayanların, çiftçilerden daha organikfosfat etkilerine daha fazla maruz kaldığını göstermektedir.

***Anahtar kelimeler**: Çiftçi, bağışıklığı baskılanmış, organofosfat maruziyeti, pestisid uygulayıcısı*

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INTRODUCTION

Pesticide is a class of environmental toxicants which hasinsecticides, herbicides and fungicides as its major subclasses. Prominent insecticide families include organochlorines, organophosphates, and carbamates. Organophosphates are quite toxic to vertebrates, and have in some cases been replaced by less toxic carbamates (1). Cholinesterase is an enzyme that is required for the proper functioning of the nervous system (2). Human exposure to OP pesticides results in inhibition of acetyl cholinesterase (AChE) and eventual accumulation of acetylcholine which interferes with muscular responses and may induce symptoms ranging from increase salivation, headache, convulsion and suppressed breathing which can result to death (3, 4). Chronic exposure to pesticides has been linked to aplastic anaemia, agranulocytosis neutropenia, thrombocytopenia (5), chronic lymphoid leukemia and multiple myeloma among farm workers (6). Although toxicologic and epidemiologic studies have demonstrated association between OP exposures and adverse health effects on nervous and reproductive systems (7, 8), the establishment of the relationship between immunological and haematological impairments in humans is far less concrete. Studies suggest immunosuppression, hypersensitivity and autoimmunity as effects of exposure to pesticides on immune system. Experimental studies have also demonstrated pesticide-induced suppression of normal immune responses to pathogens however; the mechanism of this pesticide-induced immunosuppression is still poorly understood (9).

Studies in Pakistan documented the presence and effects of pesticide residues in the blood of Karachi people (10, 11). Cruz et al. (12) also reported the presence of pesticide residue in an urban and two rural populations in Portugal. Also, Ohayo-Mitoko (13) reported occupational pesticide exposure among Kenyan agricultural workers with a view to developing strategies for the prevention and control of pesticide poisoning. In 2010, Kachaiyaphum et al.(14) reported high prevalence of abnormal serum cholinesterase levels and associated factors as well as the most common pesticide-related symptoms among Chilli-farm workers in Thailand. In Nigeria, Ivbijaro (15) evaluated insecticide residue in Kolanut farmers. Also, Sosan et al. (16) examined insecticide residue in the blood and domestic water sources, determined erythrocyte cholinesterase enzyme activity and haemoglobin values of cocoa farmers in southwestern Nigerian. Another study conducted in Taraba, Nigeria identified and evaluated pesticide urinary metabolite among agrochemical retailers (17). Majority of these previous studies concentrated on the effect of organophosphate (OP) pesticides on the activity of AChE with little attention on its effects on the haematological and immune responses in OP applicators and farmers. Due to lack of information, this present study determined haematological and immunological indices in 2 groups of Nigerian farm workers exposed to OP pesticides with a view to providing information that could be useful in providing necessary health intervention.

MATERIALS AND METHODS

After obtaining informed consent from each subject and an approval from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethical committee, 60 farm workers (47±17 years) and 30 age and sex matched civil servants who neither spray OP nor practice farming who served as controls (46±10 years) were recruited into this study. The farmworkers were further divided into two groups consisting 30 pesticide applicators (PA) and 30 farmers. About 10 ml of venous blood was obtained from each participant and 2 ml was dispensed intoEDTA bottles for the determination of complete blood count as described by Cheesbrough (18). The remaining 8 ml was dispensed into plain sample bottles to obtain serum which was stored at -200C until analyzed. Serum level of IgE was determined using ELISAas described by the manufacturer (Leinco Technology, USA) while IgA, IgM and IgG were determined using immunoplates as previously described (19). Serum activity of AChE was assayed using High Performance Liquid Chromatography (HPLC) while Skin prick test was carried out using eight allergens as previously described (20).

Statistical Analysis

The difference in means of the different groups was determined using the Student's t-test while the relationship between variables was determined using Pearson correlation coefficient. All the statistical analyses were done using the SPSS, version 17.0 and p-values less than 0.05 were considered significant.

RESULTS

In Table 1, serum AChE activity, mean PCV level, total

***Table 1**. Acetylcholinesterase (AChE) activity and haematological indices in pesticides applicators (PA), farmers exposed to organophosphate pesticides and controls.*

	Controls (n:30)	PA (n:30)	Farmers (n:30)
AChE (IU/ml)	9.38±0.82	6.63±0.90*#	7.88±0.63*
PCV (%)	42.00±3.00	40.00±4.00*	40.00±4.00*
TWBC (c/mm3)	5,210.00±1,257.00	2,890.00±794.00*	2,783.00±893.00*
Neutrophil (%)	57.00±6.00	40.00±7.00*	41.00±10.00*
Lymphocyte (%)	41.00±5.00	56.00±7.00*	56.00±8.00*
Monocyte (%)	0.93±0.17	1.53±0.19*#	0.63±0.15
Eosinophil (%)	0.63±0.11	2.23±0.31*	1.43±0.31*

*Significantly different from controls, #Significantly different from farmers

white blood cell count (TWBC) and neutrophil count were significantly reduced while the mean lymphocyte count and eosinophil count were significantly raised in both pesticides applicators (PA) and farmers compared with controls. However, mean monocyte count was significantly raised in PA compared with controls. Pesticides applicators (PA) have significantly raised mean monocyte count and significantly reduced AChE activity compared with farmers. The mean IgE level was significantly raised in both PA and farmers compared with controls. There were significantlyreduced skin prick diameters to grass and mold allergens in PA compared with controls but farmers also had significantly reduced skin prick diameter with grass allergen compared with controls. Only the mean IgM level was significantly reduced in PA compared with farmers (Table 2). In Table 3, mean serum activity of AChE had negative correlation with duration of exposure in PA. In farmers, the mean lymphocyte count had positive correlation with AChE activity whereas eosinophil, IgE and IgA levels had negative correlation with AChE activity in farmers (Table 4). As shown in Table 5, farm workers reported one or more symptoms potentially associated with exposures to pesticides. Burning sensation in the eyes/face, chest symptoms(including cold symptoms, dyspnea, chest pain), and dizziness were the most prevalent symptoms in PA whereas abdominal pain/diarrhea, chest symptoms and fever were the most prevalent in farmers.

DISCUSSION

After the flagging off of Nigerian green revolution in 1977-78; the use of pesticides in Nigeria increased thousand folds (21). Pesticides importation increased steadily from about 13 million dollars in 2001 to 28 million dollars in 2003 with insecticide accounting for about 32% of the imports (22). As agricultural production advances from subsistence to large scale farming, a concomitant increase in pesticides usage was inevitable. These however, led to the uncontrolled use and handling of pesticides leading to undesirable side effects (22).

The use of pesticides in modern agriculture improves yield, increases farmers income and government revenue as well as assured save food supply globally (23).

***Table 2**. Levels of immunoglobulin classes and skin prick diameters in pesticides applicators (PA), farmers exposed to organophosphate pesticides and controls.*

	Controls (n:30)	PA (n:30)	Farmers (n:30)
IgE (IU/ml)	229.33±178.40	320.67±172.83*	342.00±170.67*
IgA (g/L)	1.78±0.58	1.48±0.46	1.53±0.57
IgM (g/L)	0.88±0.15	0.63±0.08#	1.08±0.16
IgG (g/L)	7.57±2.52	7.73±2.50	7.94±3.30
Grass (mm)	3.27±0.18	3.00±0.13*	3.07±0.14*
Mold (mm)	4.00±0.17	3.03±0.12*	3.03±0.13 *
Dog (mm)	2.60±0.15	2.53±0.14	2.50±0.10
Mite (mm)	3.00±0.18	2.70±0.13	2.77±0.14
Cockroach (mm)	2.70±0.16	2.60±0.15	2.58±0.13
Mango (mm)	2.80±0.15	2.83±0.16	2.87±0.18
Mouse (mm)	2.88±0.16	2.94±0.18	2.92±0.14
Cat (mm)	2.59±0.17	2.55±0.14	2.53±0.12

Significantly different from controls, #Significantly different from farmers

***Table 3**. Correlation of AChE activity with duration of exposure, haematological and immunological indices in pesticide applicators exposed to organophosphatepesticides.*

	r-value	p-value
Duration of Exposure [DOE] (yrs)	-0.54	0.002*
PCV (%)	0.10	0.584
TWBC (cells/mm3)	0.12	0.513
Neutrophil (%)	-0.11	0.561
Lymphocyte (%)	0.13	0.505
Monocyte (%)	-0.02	0.898
Eosinophil (%)	-0.08	0.671
Basophil (%)	0.07	0.714
IgE (IU/ml)	-0.03	0.877
IgA (g/L)	-0.14	0.453
IgM (g/L)	-0.24	0.198
IgG (g/L)	-0.09	0.624
Grass (mm)	-0.17	0.366
Mold (mm)	0.16	0.402
Dog (mm)	0.18	0.325
Mite (mm)	0.17	0.386
Cockroach (mm)	0.16	0.190
Mango (mm)	0.19	0.200
Mouse (mm)	0.20	0.085
Cat (mm)	0.22	0.075

**Significant at P<0.05 (2-tailed)*

***Table 4**. Correlation of AChE activity with duration of exposure, haematological and immunological indices in farmers exposed to organophosphatepesticides.*

	r-value	p-value
Duration of exposure(yrs)	-0.12	0.547
PCV (%)	-0.05	0.803
TWBC (cells/mm3)	0.22	0.235
Neutrophil (%)	-0.21	0.260
Lymphocyte (%)	0.36	0.048*
Monocyte (%)	0.22	0.245
Eosinophil (%)	-0.39	0.032*
IgE (IU/ml)	-0.45	0.013*
IgA (g/L)	-0.47	0.010*
IgM (g/L)	0.04	0.776
IgG (g/L)	-0.08	0.677
Grass (mm)	-0.15	0.419
Mold (mm)	0.28	0.140
Dog (mm)	0.16	0.243
Mite (mm)	0.23	0.226
Cockroach (mm)	0.19	0.201
Mango (mm)	0.20	0.091
Mouse (mm)	0.24	0.199
Cat (mm)	0.20	0239

**Significant at P<0.05 (2-tailed)*

In recent years however, there has been increasing concern regarding potential adverse effects of pesticides on occupationally exposed individuals (24).

In this study, AChE activity was significantly lower in pesticides applicators (PA) and farmers compared with the controls. This observation supports earlier reports (25, 26). Exposure to OP pesticides exerts toxic effect by inhibiting enzymatic degradation of neurotransmitter; acetylcholine which results inaccumulation of acetylcholine, continued excitation of the neurons, paralysis, respiratory failure or death (27). This necessitated its use as an index of chronic exposure to OP in farmworkers.Surprisingly, the activity of AChE was significantly lower in pesticides applicators (PA) compared with farmers. This indicates that pesticides applicators are at higher risk of developing health risks associated with OP exposure including pesticide-induced immunosuppression. Our observed significant reduction in mean PCV, TWBC, neutrophil count andsignificant elevation in lymphocyte and eosinophil counts in both PA and farmers compared with controls is consistent with the report of Desi (28). Edem et al. (29) also reported a similar observation in experimental animals. The reduced PCV observed could be suggestive of microcytic anaemia. Since AChE is also found in human red blood cells (RBCs) (30), reduced PCV could also lead to reduced AChE activity observed in the farm workers. However, monocyte count was significantly elevated in pesticides applicators (PA) compared with controls and farmers. Our observation could indicate chemical stress as suggested by Al-Sarar et al. (31) and Azmi et al. (32) or even, monocytic leukemia (6). This observation, in line with the earlier reports, indicates that monocytosis is a feature of chronic exposure to organophosphate (OP) pesticides. Immunosuppression, hypersensistivity and autoimmunity have been reported as effects of OP pesticides on the immune system (9). Significant elevation of serum IgE and eosinophil observed in both PA and farmers compared with control indicates that OP could act as an allergen. Elevated serum IgE has been reported in cement factory workers (33) and in subjects with asthma (34). It is therefore not surprising that burning sensations, cough, skin rashes were common toxicity symptoms in our subjects (Table 5). These symptoms, together with the observed elevated IgE level and eosinophilia count suggest allergic reactions which might indicate that long term inhalation of OP may predispose the PA and farmers to asthmatic attack.

Serum IgM level was significantly lower in PA compared with the farmers. This probably, suggests secondary selective immunoglobulin M (SIgM) deficiency which is usually associated with malignancy, autoimmune disease,

***Table 5**. Prevalence of toxicity symptoms among farm workers exposed to organophosphate pesticides*

	Applicators (n:30)		**Farmers (n:30)**	
Symptoms	**Number**	**%**	**Number**	**%**
Headache	10	33.3	10	33.3
Burning sensation in eyes/face	30	100	05	16.6
Weakness	15	50	18	60
Fever	20	66.6	21	70
Skin rash	25	83.3	10	33.3
Itching and skin irritation	22	73.3	12	40
Dizziness	26	86.6	20	66.6
Chest symptoms, including chest pain	28	93.3	21	70
Excessive sweating	12	40	18	60
Salivation, nausea and vomiting	08	26.6	05	16.6
Abdominal pain /diarrhea	25	83.3	22	73.3

gastrointestinal disease, and immunosuppressive treatment (35). Our observation probably indicates that PA are at risk of developing diseases associated with selective IgM deficiency. The significant inverse correlation observed between AChE activity and duration of exposure in PA indicates that AChE activity reduces progressively with increasing number of years of OP exposure. In the farmers, AChE activity had an inverse correlation with IgE level and eosinophil suggesting that farmers might be at higher risk of developing allergic rhinitis and asthma since these diseases are IgE mediated (36). However, further work is required to find out if elevated serum IgE level and oesinophil count could be used as indices of OP exposure like AChE activity.

Skin prick testing is conventionally used to investigate immediate type hypersensitivity to allergens. It is also a means of detecting allergen specific IgE and has the advantage of being relatively inexpensive, providing immediate results compared with measurement of serum allergen specific IgE by radioallergosorbent testing (37). In this study, farmworkers have reduced skin reaction diameters to grass and mold allergens compared to controls. Our observation could indicate immune tolerance to these allergens due to persistent exposure. A reduced skin diameter does not mean that the subject is not allergic to a substance but the body might fail to elicit enough response as a result of continuous contact (38). The implication of reduced skin prick diameters in farmworkers is that this methodology may not be useful in assessing immediate hypersensitivity status in them.

It could be concluded from this study that increased monocyte count and reduced IgM level may differentiate pesticide applicators from farmers exposed to OP and that pesticide applicators are more prone to immunologic effects of organophospahte pesticides than farmers.

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