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Original Article

Serum Levels of Selected Cytokines in Male Nigerian Farm Workers Exposed to Dichlorvos Organophosphate Pesticide

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Abstract: Background: Dichlorvos Organophosphate Pesticide (DOP) is an occupational and environmental toxicant which has been classified as highly hazardous and carcinogenic. DOP has been associated with a number of immunocompromised effects as it interferes and interacts with the specific immunological functions. However, there is lack of information on the effects of DOP on serum cytokine levels in DOP users, thus, selected pro- and anti-inflammatory cytokines were therefore measured in adult male farmer workers exposed to DOP. Materials and Methods: One hundred and twenty adult male Farm Workers (FW) consisting of 60 Pesticide Applicators (PA) and 60 farmers exposed to DOP for not less than ten years were randomly enrolled into this study. Sixty apparently healthy adult males without occupational exposure to DOP served as controls. Serum levels of pro-inflammatory cytokines [tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ)] and anti-inflammatory cytokines [interleukins (IL)-4 and IL-10] were determined using ELISA. Data were analyzed using ANOVA, Post-Hoc and Pearson correlation coefficient. P-values less than 0.05 were considered as statistically significant. Results: The serum levels of IFN-γ and IL-4 were significantly higher in FW compared with the controls while the serum levels of IFN-γ, IL-4 and IL-10 were significantly higher in PA compared with the farmers. IL-4 had significant positive correlation with IFN-ϒ in farm workers, PA and farmers exposed to DOP but IL-4 had significant positive correlation with TNF-α in farmers only. Conclusion: There is a balanced pro and anti-inflammatory cytokine secretion in farm workers with long term exposure to DOP which regulates the pathogenicity of DOP exposure.

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INTRODUCTION

Dichlorvos (Dimethyl 2, 2-dichlorovinyl phosphate, DDVP) belongs to organophosphate family of insecticide pesticides. It is classified as a group Ib and IIb chemical, reflecting its highly hazardous and carcinogenic effects on humans exposed (IPCS, 2010; WHO, 2020). The main release route of DOP is through emission during agricultural and industrial uses and enters the body of exposed individuals through inhalation, dermal contact and ingestion (Chandra et al., 2017; Costa, 2018).

Previous human and experimental studies have established neurotoxic effect of exposure to DOP through inhibition of acetylcholinesterase (AChE) by binding covalently to AChE active site (Galloway and Handy, 2003; Gupta, 2006; Costa, 2018). The inhibition of AChE by DOP causes accumulation of enormous acetylcholine in the synapses which is responsible for toxicity of the neurons manifested as muscle paralysis and organophosphate-induced delayed polyneuropathy (Gupta, 2006).

Apart from the effect of DOP on the nervous system, experimental studies proposed toxic effects of DOP on immune and endocrine systems which depended on the dose and duration of exposure (Sarwa, 2015; Gangemi et al., 2016). However, the impact of chronic exposure on cytokine levels is still unclear.

TNF-α and INF-ϒ are pro-inflammatory cytokines whose levels are increased in various clinical conditions whereas IL-4 is an anti-inflammatory cytokine which enhances humoral immunity by increasing the number of Th2 cells (Arinola, 2016). IL-10 is an essential pleiotropic immune regulator cytokine mainly secreted by macrophages. It is a centrally operating anti-inflammatory cytokine (Van excel et al., 2010). Its immunosuppressive effects protect humans from exaggerated inflammatory responses and autoimmune diseases (Schottelius et al., 1999). Interaction and interference of DOP with the cytokine receptor sites lead to modulation of signal transduction pathways that control proliferation and differentiation of immune cells (Luster et al., 1992). The DOP-cytokine interaction may reduce or enhance cytokine production by the activated immune cells. Thus, the effects of DOP on the activated immunocytes can be expressed as either stimulatory enhancing cytokine production which can result in hypersensitivity or autoimmunity or inhibitory suppressing cytokine production which can result in increased susceptibility of the host to infectious and neoplastic agents.

Dichlorvos is commonly used in many agrarian communities in Nigeria. It is traded under different trade names such as DDforce, Siege, Snipers, Didwell among others. Although DOP exposure has been associated with a number of pathological conditions, information on the possible effects of DOP exposure on pattern of cytokine secretion in Nigerians with long term exposure to DOP is scarce. This thus serves as the basis for this study.

MATERIALS AND METHODS

Study participants

A total of 120 adult male farm workers (47 ± 7 years) and 60 age and sex matched civil servants (46 ± 10 years) that neither use DOP nor practice farming who served as controls were enrolled into this study. The farm workers were further divided into two groups consisting 60 pesticide applicators (PA) and 60 farmers.

Ethical consideration

Approval was obtained from the University of Ibadan/University College Hospital Joint Ethics Committee (UI/EC/11/0107). Also, written informed consent was obtained from each participant.

Blood sample collection and laboratory analyses

Venous blood (5 ml) was obtained from each study participant and dispensed into plain sample bottles to obtain serum which was stored at -20ºC until analyzed. The serum levels of pro-inflammatory cytokines [Tumour Necrosis Factor-α (TNF-α) and Interferon gamma (IFN-γ)] and anti-inflammatory cytokines [Interleukins (IL-4) and (IL-10)] were determined using ELISA as described by the manufacturer (Elabscience Biotechnology Company Ltd. USA).

Statistical analyses

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 median (interquartile range).

RESULTS

As shown in Table 1, the median serum levels of IFN-γ (p = 0.001) and IL-4 (p = 0.001) were significantly higher in farm workers compared with the controls. However, the serum levels of IL-10 (p = 0.952) and TNF-α (p = 0.180) were not significantly different between the 2 groups.

Table 1: Serum levels of selected cytokines in farm workers exposed to DOP and controls

	Controls (n = 60)	Farm workers (n = 120)	P-value
TNF-α (pg/mL)	15.0(15.0-19.0)	16.0(15.0-21.0)	0.180
IFN-γ (pg/mL)	21.7(12.1-50.5)	71.7(42.4-119.2)	<0.001*
IL-4 (pg/mL)	58.7(15.2-154.2)	165(103-246.5)	<0.001*
IL-10 (pg/mL)	95.0(37.0-157.0)	75(48.0-144.0)	0.952

*Significantly different from controls at P < 0.05
TNF-α = Tumuor Necrosis Factor-alpha, IFN-γ = Interferon gamma, IL-4 = Interleukin-4, IL-10 = Interleukin-10

Similarly, the median serum levels of IFN-γ (p =0.001) and IL-4 (p = 0.001) were significantly higher in PA compared with the controls and in farmers compared with the controls (Table 2).

Table 2: Serum levels of selected cytokines in pesticide applicators, farmers and controls

	Controls (n = 60)	Pesticide Applicators (n = 60)	Farmers (n = 60)	P-value[a]	P-value[b]
TNF-α (pg/mL)	15.0 (15.0 - 19.0)	16.0 (15.0 - 21.0)	15.0 (15.0 - 20.0)	0.185	0.302
IFN-γ (pg/mL)	21.7 (12.1 - 50.5)	94 (50.4 - 119.7)	62.6 (39.9 - 100.0)	<0.001*	<0.001*
IL-4 (pg/mL)	58.7(15.2 - 154.2)	177 (121 - 262.1)	137 (87.6 - 233.9)	<0.001*	<0.001*
IL-10 (pg/mL)	95.0(37.0 - 157.0)	104(52.0 - 148.0)	60 (48.0 - 115.0)	0.303	0.256

*Significant at *P* < 0.05, [a]Pesticide applicators vs controls, [b]farmers vs controls
TNF-α = Tumuor Necrosis Factor-alpha, IFN-γ = Interferon gamma, IL-4 = Interleukin-4, IL-10 = Interleukin-1

Comparing PA with the farmers, it was observed that the median levels of IFN-γ (p = 0.026), IL-4 (p = 0.018) and IL-10 (p = 0.025) were significantly higher in PA compared with the farmers (Table 3).

As shown in Table 4, IL-4 had significant positive correlation with IFN-ϒ (r = 0.949, p = 0.000) in farm workers, PA and farmers exposed to DOP but IL-4 had significant positive correlation with TNF-α (r = 0.267, p = 0.039) in farmers only. No significant correlation was observed within other cytokines.

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

	Pesticide Applicators (n = 60)	Farmers (n = 60)	*P*-value
TNF-α (pg/mL)	16.0 (15.0 - 21.0)	15.0 (15.0 - 20.0)	0.762
IFN-γ (pg/mL)	94 (50.4 - 119.7)	62.6 (39.9 - 100.0)	0.026*
IL-4 (pg/mL)	177 (121 - 262.1)	137 (87.6 - 233.9)	0.018*
IL-10 (pg/mL)	104 (52.0 - 148.0)	60 (48.0 - 115.0)	0.025*

*Significant from farmers at *P* < 0.05.
TNF-α = Tumuor Necrosis Factor-alpha, IFN-γ = Interferon gamma, IL-4 = Interleukin-4, IL-10 = Interleukin-10

DISCUSSION

Although previous studies have shown that DOP exposure has been associated with a number of pathological conditions including profound alterations in immune responses which are defined by slow evolution and long-term duration (Edem et al., 2012; Costal et al., 2013; Gangemi et al., 2016). However, information on the possible effects of DOP exposure on pattern of cytokine secretion in male Nigerians farm workers with long term exposure to DOP is scarce.

Cytokines are essential in regulating immune responses in humans. Therefore, the understanding of homeostasis between pro- and anti-inflammatory cytokines is important in the delineation of pathological conditions that may ensue from the imbalance of cytokine homeostasis (Gangemi et al., 2016). Alteration in cytokine homeostasis has been implicated in the pathogenesis of a number of infectious diseases (Giamarellos-bourboulis et al., 2020; McKechnie and Blish, 2020) and pesticide exposure (Gangemi et al., 2016).

Previous reports showed that some xenobiotics that block the biological activities of cytokines may either bind directly to a cytokine receptor on the immunocyte without activating them (or over activate them) or inhibiting the cytokine activity itself by binding directly to the cytokine since they are protein (Newcombe and Esa, 1992a). A number of cytokine mimics referred to as cytokine-binding chemicals may enhance or reduce the cytokins' inflammatory effects are solube in nature (Morgan, 1992; Akdis et al., 2011). It is possible that dichlorvos or its metabolites either bind directly to cytokine receptor on immune cell or directly to the cytokine itself thereby inhibiting or enhancing its activity.

The observed significantly higher serum levels of IFN-γ and IL-4 in farm workers, PA and farmers exposed to DOP compared with the controls contradicts the report of Costa *et al*. 2013 who reported reduced levels of pro-inflammatory cytokines in greenhouse workers exposed to pesticides. The elevated levels of cytokines in our study may suggest that prolonged exposure to DOP by farm workers resulted in interaction of pesticide residues or its metabolites with cytokine receptors on the immune cells. Activated macrophages promotes the development of Th1 cells by stimulating IFN-γ production, an important pro-inflammatory cytokines. The activation of macrophages by IFN-γ is accompanied by increased expression of the Fc receptors of immunoglobulin (FcR) which promotes phagocytosis of immune complexes and increases the capacity of the macrophages to lyse antibody-coated bacteria, parasites and tumour cells by ADCC (Kunkel and Butcher, 2002).

Table 4: Correlation within cytokines in farm workers, pesticide applicators and farmers exposed to DOP

Parameters	TNF-α r-value	P-value	IL-10 r-value	p-value	IL-4 r-value	p-value	IFN-ϒ r-value	p-value
Farm workers								
TNFα(pg/ml)	-	-	-0.103	0.262	0.072	0.434	0.041	0.657
IL-10 (pg/ml)	-0.103	0.262	-	-	0.014	0.876	0.040	0.665
IL-4 (pg/ml)	0.072	0.434	0.014	0.876	-	-	0.949	0.000*
IFNϒ(pg/ml)	0.041	0.657	0.040	0.665	0.949	0.000*	-	-
PesticideApplicators								
TNFα(pg/ml)	-	-	0.014	0.913	-0.106	0.418	-0.100	0.446
IL-10 (pg/ml)	0.014	0.913	-	-	-0.088	0.505	-0.037	0.778
IL-4 (pg/ml)	-0.106	0.418	-0.088	0.505	-	-	0.959	0.000*
IFNϒ(pg/ml)	-0.100	0.446	-0.037	0.778	0.959	0.000*	-	-
Farmers								
TNFα(pg/ml)	-	-	-0.125	0.340	0.267	0.039*	0.242	0.063
IL-10 (pg/ml)	-0.125	0.340	-	-	0.092	0.486	0.087	0.510
IL-4 (pg/ml)	0.267	0.039*	0.092	0.486	-	-	0.914	0.000*
IFNϒ(pg/ml)	0.242	0.063	0.087	0.510	0.914	0.000*	-	-

Values are reported as correlation coefficient, P-value:
*Significantly correlated at *P*<0.05 (2-tailed)
TNF-α = Tumuor Necrosis Factor-alpha, IFN-γ = Interferon gamma, IL-4 = Interleukin-4, IL-10 = interleukin-10

On the other hand, IL-4 promotes Th2 cell differentiation. It is produced primarily by mast cells, Th2 cells, eosinophils and basophils (Choi and Reieser, 1998). In this study, elevated serum level of IL-4 in farm workers, PA and farmers indicates the involvement of Th2 cells in immune response against exposure to DOP. IL-4 induces the differentiation of naive helper T cells (Th0 cells) into Th2 cells. Upon activation by IL-4, Th2 cells subsequently produce additional IL-4 in a positive feedback loop. Also, IL-4 drives the immunoglobulin (Ig) class switch to IgG1 and IgE. Moreover, IL-4 induces alternative macrophage activation and up-regulates MHC class II production (Choi and Reiser, 1998). Thus, increased IL-4 levels in our study might be a physiologic response to balance observed elevated Th1 cytokines in farm workers.

Comparing exposed farm workers with the controls, the increased pro (IFN-γ) and anti (IL-4 and IL-10) inflammatory cytokines production might prone them to immune related pathologies induced by cytokine imbalance. Since cytokine environment determines the subsequent development of the adaptive immune response i.e. to predominantly Th1 or Th2 (Abbas et al., 2017), this observation showed that both Th1 and Th2 pathways are activated in response to exposure to DOP by farm workers, PA and farmers. This was further alluded to by a significant positive correlation between IL-4 and IFN-ϒ in the exposed workers indicating cytokine homeostasis in long term DOP exposure. Robbe *et al*. 2014 reported a shift in T-cell polarisation to Th1 following agricultural dust exposure in mice and men.

Comparing pesticide applicators and farmers, the observed significant elevation in the serum median levels of IFN-γ, IL-4 and IL-10 in PA compared with the farmers suggests heightened cytokine production in PA than farmers which might assume higher anti-inflammatory responses in PA compared with the farmers. This observation supports the dose-effect relationship earlier postulated (Costa, 2018).

The observed elevation in the serum level of IFN-γ in PA is probably counter balanced by a concordant increase in the level of anti-inflammatory cytokines; IL-4 and IL-10. The observed elevation in IL-10 level could be a physiologic response to down-regulate the inflammation in PA induced by elevated IFN-γ which might explain why these workers are protected despite regular and prolonged exposure. The significant positive correlation observed between IL-4 and TNF-α in farmers might differentiate the cytokine profile of PA and farmers. Thus, IL-4 had significant positive correlation with both IFN-ϒ and TNF-α in farmers whereas IL-4 showed significant positive correlation with IFN-ϒ only in PA.

It can be concluded from this study that there is a balanced pro and anti-inflammatory cytokine secretion in farm workers with long term exposure to DOP which regulates the pathogenicity of DOP exposure.

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Conflict of interest

Authors have no conflict of interest to declare

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