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Pesticide-induced Biochemical and Toxicological Changes in Farmers in a South-East Metropolis of Nigeria

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Abstract: Pesticides are widely used for crop protection to increase crop yields. However, they can have significant adverse effects on farmers and possibly consumers. These effects result from the use of inappropriate spraying equipment and the failure to use personal protective equipment, especially in developing countries. This study aimed to evaluate biochemical changes in farmers who use pesticides in the Enugu metropolis of Nigeria and reasoned that if crops can assimilate pesticides from the soil, consumers may also experience similar adverse effects. The cross-sectional study involved 150 subsistence farmers in Enugu Metropolis of Enugu State, Nigeria, and 120 age-matched office workers who were not involved in farming. The biochemical parameters evaluated included lipid profiles (total cholesterol, high-density cholesterol, low-density cholesterol, very low-density cholesterol and triglycerides), electrolytes, urea, creatinine and liver enzymes (alkaline phosphatase, aspartate transaminase and alanine transaminase). All parameters were measured spectrophotometrically, except electrolytes determined with ion-selective electrodes. SPSS was used to analyze the data, with a significant value of $p \leq 0.05$. The results showed that total cholesterol, low-density lipoprotein, very low-density lipoprotein and triglycerides increased significantly in pesticide-exposed persons compared to non-exposed persons (controls). Also significantly increased in farmers were urea concentration and alanine transaminase activities. There were no significant differences in concentrations of high-density lipoprotein, creatinine, electrolytes, and aspartate transaminase activity. These results implied that pesticides can cause dyslipidemia, subclinical renal disturbances and hepatotoxicity, calling for appropriate health education for farmers and insistence on the use of personal protective equipment.

Keywords: Pesticide toxicity, Biochemical changes, Farmers, Consumers

INTRODUCTION

Pesticides are widely used in agriculture to control pests and diseases. They are easily available and therefore commonly used in the rural areas of developing countries. These pesticides include diclorvos-diphenyl trichloroethane (DDT), organophosphates (OPs), carbamates (CMs) and their derivatives. Control of pests helps to increase agricultural yields; however, exposure to these pesticides can have severe health implications for crop farmers [1]. Unfortunately, most farmers using these pesticides are not fully aware of the side effects of these chemicals on their health, probably due to a lack of or low educational levels of these farmers, especially in developing countries. In developing countries, farmers are exposed to increasing amounts of pesticide mixtures at high concentrations and frequencies, including even those banned in industrialized countries. These adverse effects range from skin rash, facial burns, and eye irritation to possible long-term effects such as developmental toxicity and neurotoxicity, as well as pathophysiological actions that have not yet been described [2]. Because of poor knowledge of the adverse effects of these pesticides, farmers generally discard their pesticide containers in the bushes or farms, or wash them into nearby streams (endangering aquatic life). When they are brought home, they are littered in the vicinity of their homes, exposing family members and neighbours to the chemicals, either by inhalation or ingestion through contaminated utensils, water, and food; thus making non-farmers liable to the same effects as the exposed [3]. Inappropriate handling of these containers contravenes the recommended practice of either incineration (depending on the chemical class) or recycling of the pesticide containers by the producers [4, 5].

The liver is the major bio-transformative organ in the body, and thus, a good defence system against environmental toxicants (xenobiotics) and metabolic toxins [2]. Previous results revealed a significant increase in ALT in agro-pesticide users, while AST remained unchanged [6-8]. Likewise, insecticides and fungicides were found to be hepatotoxic in rodents [9-11]. The kidney, which is involved in the elimination of toxic compounds from the body, is also affected by pesticides. Ingestion or inhalation of glyphosates (a class of OP) has been found to alter serum creatinine and glomerular filtration rate in farmers, and to cause both liver and kidney damage in rats [12]. Furthermore, some pesticides have been described as endocrine disruptors due to their effects on estrogenic and androgenic nuclear receptors [13]. The recent rise in the use of Organochlorine pesticides (OCPs) in Nigeria may be associated with the risk of metabolic syndrome, a precursor of diabetes [14]. Metabolic syndrome includes several disease conditions like insulin resistance, dyslipidemia, hypertension and overweight, all of which are connected to cardiovascular disease. Long-term exposure to pesticides (use or consumption of vegetables grown with them) has been suspected to cause chronic diseases such as neurotoxicity, cancer, necrosis, asthma, reproductive disorder, cardiac disease, and diabetes [15]. While carbamates inhibit acetylcholinesterase (AChE) activity, causing lacrimation and neurotoxicity [8, 16], organophosphorus (malathion and parathion) facilitate cellular growth and proliferation, leading to cancer, especially breast cancer and also reduce paraoxonase activity, increasing the risk of coronary artery disease [17, 18]. Other cholinergic symptoms include cramps, increased salivation, muscle weakness, paralysis, diarrhea, and blurred vision [19]. Low cholinesterase activity is known to cause hyperlipidemia, indicating that pesticides might cause an increase in serum lipids, leading to cardiovascular disease.

The majority of pesticide users in Enugu metropolis are subsistence farmers who take to farming to provide for their immediate families, while making paltry earnings. For this, many of them access the chemicals from local vendors and retailers with little or no knowledge of the chemical constituents or the dangers they pose. In Nigeria, in particular, Ops are sold directly from shops due to the lack of specific rules and regulations governing their use and sale [1]. Furthermore, many farmers fail to read the pesticide label before application, and hence might not follow the recommended doses. Therefore, some active ingredients, already banned in Nigeria and beyond [20], are among the agro-pesticides used by the farmers. Major exposure to pesticide chemicals occurs through the mouth (ingestion), nose (inhalation), and skin (dermal contact). Therefore, these routes should be effectively protected by the use of appropriate personal protective equipment (PPE) such as masks, gloves, and overalls. Unfortunately, these subsistence farmers hardly use gloves or face masks. Their clothes are mainly of cotton or permeable materials, which permit pesticide suspensions to pass through the clothes and contact the skin. Unfortunately, inappropriate or absence of protective tools during pesticide application is known to contribute significantly to pesticide exposure [2], resulting in immediately observable symptoms such as skin rash, facial burn, and eye irritation, as well as respiratory symptoms for highly volatile pesticides [21]. There is a dearth of information on the effects of these pesticides on farmers within the Enugu metropolis of Enugu State, Nigeria. This study aimed to evaluate the impact of these pesticides on farmers, who are mainly subsistence farmers. The results will help governments, at all levels, regulate the use of pesticides and encourage farmers to adopt healthy measures, like the use of PPE, to save their lives.

METHODS

Ethical clearance: Ethical clearance for this study was obtained from the Ethical Clearance Committee of the Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology, Enugu, Nigeria, while oral informed consents were sought and obtained from the participants after a detailed explanation of the procedures for the study.

Study area: Enugu metropolis is the capital of Enugu State, Nigeria. The state lies between latitudes 5 ° 56 ' N and 7 ° 55 ' N, and longitude 6 °53 ' E and 7 °55 ' E, with a total land area of 802,295km and a population density of 248 persons/K^2, while Enugu metropolis has an estimated population of 907,000 in 2025 and a growth rate of 3.54% [22, 23]. The area is populated by civil servants of various classes and middle-class traders, making it prone to subsistence farming. The farming period is mainly during the dry season, and at river banks, using local irrigation.

Study population: This is a cross-sectional study involving 150 civil servants, traders and artisans who use pesticides in their subsistence farming within the metropolis. Subsistence farming is employed to meet the family's needs, and a little for paltry earnings. Controls were 120 age and sex-matched office workers who were not involved in any form of farming. All subjects were between 18 and 55 years of age.

Sample size calculation: Sample size was calculated using Cochran's formula [24]

$$n = Z^2p(1-p)/e^2$$

where:

- n = minimum sample size.
- Z = standard normal deviation for 95% confidence (1.96).
- p = estimated proportion of farmers in Enugu metropolis (10%).
- e = margin of error (5% / 0.05).

$$q = 1 - p = 1 - 0.10 = 0.99$$

Therefore $n = (1.96^2 \times 0.10 \times 0.99) \div 0.05^2$

$$n = 0.3803184 \div 0.0025$$

$$n = 152$$

$$n \approx 150$$

Sample collection: A total of 5.0ml of blood was collected from each subject into a grease-free, glass test tube. This was allowed to stand at room temperature for ten minutes, to clot and retract. It was then centrifuged at 5000 rpm for ten minutes. The resultant serum was then preserved in the freezer at -20 °C until needed for analysis.

Biochemical analyses: concentrations of lipid profile parameters and liver enzymes' activities were determined as previously reported [25, 26], while urea and creatinine concentrations were estimated using the enzymatic method and the Jaffe reaction, respectively, as previously reported [27, 28]. Electrolytes were determined using ion-selective electrodes.

Statistical analysis: The data obtained were analyzed using the GraphPad Prism version 10. Continuous variables were presented as means and standard deviations, with a statistically significant level set at $p \leq 0.05$.

RESULTS

Table 1 shows the demographic characteristics of the subjects. It shows that 58% were male, while 42% were female. For age distribution, 23 (16.5%) of them were between 18-25 years, 38 (29.0%) between 26-35 years, 78(45.0%) between 36-45 years, and 11(9.5%) between 46-55 years.

Table 1: Socio-demographic characteristics of the study participants

Demography	Frequency	%
Gender		
Male	80	58%
female	70	42%
Age groups		
18-25	23	16.5%
26-35	38	29.0%
36-45	78	45.0%
46-55	11	9.5%
Total	150	100%
Religion		
Christianity	100	75%
Islam	40	17.5%

Traditional	10	7.5%
Total	150	100%
Education Background		
Primary School	35	25%
Secondary School	80	47.5%
Tertiary Institution	15	12.5%
None	20	15%
Total	150	100%
Marital Status		
Single	15	5%
Married	130	92.5%
Divorced	2	1%
Separated	3	1.5%
Widow/Widower	0	0%
Total	150	100%

The majority of them (75.0%) were Christians (depicting the prevalent religion in the area of study), followed by Muslims (17.5%), while the remaining 7.5% were Traditional worshippers. Based on their educational status, most of them had secondary education (47.5%), followed by primary education (25.0%), no formal education (15.0%) and lastly, tertiary education (12.5%). Most of the respondents were married (92.5%), followed by those who were single (5.0%), separated (1.5%), and divorced (1.0%).

Table 2 shows the lipid profiles of both pesticide-exposed and non-exposed subjects. The results show statistically significant differences in lipid profiles between the two groups. T-Chol, TG, LDL and VLDL from the pesticide-exposed subjects were significantly higher than those from non-exposed (control) subjects ($p<0.001$), while HDL from the exposed subjects was significantly lower than that from the control subjects ($p<0.031$).

Table 2: Comparison of lipid profiles in pesticide-exposed people and control subjects.

Variables	Exposed subjects	Control	F	p-value
T- Chol (mmol/L)	4.714±0.94	3.948±1.75	1.439	0.043*
TG (mmol/L)	1.292±0.62	0.916±0.42	1.199	0.001*
HDL (mmol/L)	1.088±0.53	1.598±0.54	1.336	0.031*
LDL (mmol/L)	3.016±0.77	1.958±1.83	1.803	0.000*
VLDL (mmol/L)	0.582±0.28	0.392±0.17	1.118	0.001*

The serum electrolytes, urea and creatinine of the exposed and control subjects are shown in Table 3.

The results showed there were no statistically significant differences in all the variables, except for the urea level, which was significantly higher in the exposed subjects than in control subjects ($p = 0.047$).

This suggests a potential association between pesticide exposure and altered nitrogen metabolism.

Table 3: Serum electrolytes, urea and creatinine in exposed and control subjects

Variables	Exposed subjects	Controls	t-value	p-value
Sodium (mmol/L)	141.15±2.78	141.75±3.09	-0.767	0.453
Potassium (mmol/L)	3.930±0.39	3.990±0.39	-0.480	0.636
Chloride (mmol/L)	101.55±1.93	101.85±1.87	-0.465	0.647
Bicarbonate (mmol/L)	25.70±1.72	26.35±1.66	-1.347	0.194
Urea (mmol/L)	4.450±1.68	3.640±1.12	2.121	0.047
Creatinine (μmol/L)	94.115±20.15	86.770±13.61	1.624	0.121

Table 4 shows the activities of the liver enzymes assayed in both exposed and control subjects. The results showed that ALT activity in exposed subjects was significantly higher than that in controls. However, there were no significant differences between the two groups in their AST and ALP activities.

Table 4: Activities of some liver enzymes in exposed and control subjects

Variables	Exposed subjects	Controls	F values	p-values
ALT (IU/L)	45.76 ± 4.82	8.88 ± 2.77	3.014	<0.001
AST (IU/L)	14.04 ± 4.95	13.96 ± 4.75	1.085	0.958
ALP (IU/L)	93.03 ± 29.78	92.38 ± 28.68	1.078	0.945

ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase

DISCUSSION

The demographic characteristics of the subjects revealed that more men are involved in subsistence farming in the metropolis. Furthermore, the active age of 36 – 45 years has the highest frequency. This indicates that newly married men and women mostly engage in this subsistence farming to provide for their new families and to save from their earnings for other financial involvements. This is supported by the finding that over 90% of the farmers are married men and women, and that the majority of them are secondary school leavers, followed by primary school leavers, implying low-income earners. The results also showed that all the lipid profile parameters increased significantly in farmers compared to controls, except HDL, which decreased significantly. Some organophosphorus compounds facilitate cellular growth and proliferation, and reduce the activity of paraoxonase, leading to cancer and increased risk of cardiovascular disease [17]. They also reduce cholinesterase activity, and decreased activity of cholinesterase is known to increase serum lipids [29]. As a class of xenobiotics, pesticides activate hormone-sensitive triglyceride lipase, which hydrolyses stored triglycerides and mobilizes free fatty acids in blood, causing hyperlipidemia [30]. Likewise, the cytotoxic effects of pesticides on pancreatic cells can cause insulin deficiency, leading to diabetes [31]. Increased energy demand during diabetes increases lipolysis, leading to hyperlipidemia. Hyperlipidemia is a significant cause of cardiovascular disease, especially with a significant increase in VLDL and triglycerides [32-34]. The assessment of the effects of pesticides on the kidneys showed no statistically significant differences between the exposed and the unexposed subjects, except for urea, which increased significantly in exposed subjects. This study is in agreement with recent studies, which demonstrated that glyphosate, a class of organophosphorus compounds, caused significant increases in blood urea levels [35-37]. Creatinine, which is a test of glomerular filtration rate, increased relatively, but non-significantly, in exposed subjects, supporting the results of an earlier study [38]. Though the urea levels in these exposed subjects did not exceed the reference values for serum urea, implying no immediate imbalance in nitrogen metabolism, the significant difference in urea levels, alongside the relatively higher creatinine levels, raises concerns about the potential for subclinical renal stress in those exposed farmers. Such subclinical stress can become obvious with time, making long-term exposed farmers liable to kidney disease. The non-significant differences in electrolytes can be attributed to the usual compensatory functions of the kidneys, geared towards maintaining the acid-base balance of the body. This study also showed alanine transaminase (ALT) significantly increased in exposed subjects compared to controls, while aspartate transaminase (AST) and alkaline phosphatase (ALP) did not show significant changes. The results are in agreement with previous studies on the effects of pesticides on the liver [6-8, 39]. The liver is the site of most biotransformation in the body, including many biochemical and toxicological metabolisms [40]. Increased activities of liver enzymes, particularly ALT and AST, are usually associated with toxicities of liver cells. These increased activities indicate altered plasma membrane permeability and tissue histology, and cellular damage [41]. Though these two enzymes are known to increase when there is liver cell damage [26], an increase in AST activity can be seen in other conditions, like some cardiovascular diseases, leaving ALT as the main indicator of hepatocellular changes. Therefore, an increase in the activity of ALT, with a relative increase in the activities of AST and ALP, is indicative of liver toxicity. Pesticides are sprayed either on crops against air-borne diseases or into the soil against earth-borne diseases. Therefore, crops can be contaminated through assimilation, either from the air during spraying or from the soil through the roots; thus, there is a possibility of such pesticides being ingested by consumers of the crops, who are not farmers. Depending on the amount of pesticides assimilated/ingested, the quantity of contaminated crops consumed and the duration of consumption, consumers can be inadvertently prone to hyperlipidemia and other adverse effects caused by these pesticides. Hence, not only are farmers affected by pesticides, but consumers of the crops grown or preserved with those pesticides. Such a situation has been observed in ethno-medicinal plants grown in heavy metal mining areas [3]. Furthermore, pesticides can affect the nutritional qualities of crops by reducing the oxidative status of crops' phytochemicals. Also, considering the modes of action of pesticides like nerve, metabolic, and muscle poisoning, as well as physical toxicity [41], there will be a plethora of effects on the consumers who assimilate them passively for a long time. The same fate befalls the family members of farmers who ingest and inhale pesticides from littered materials and consume foods grown with the pesticides.

CONCLUSION

This study showed that pesticides have significant effects on the users; there were noticeable dyslipidemia, subclinical renal stress that may express itself at a later time and liver toxicity. Therefore, subsistence farmers in developing countries should be trained to understand the side effects of these pesticides and to use personal protective equipment (PPE) when spraying them. Moreover, attention should be given to consumers of these agro-products and to farmers' family members, who are silently exposed to the effects of these pesticides and may be affected later in life.

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