Original Research Article

Evaluation of some kidney and liver function markers in humans exposed to pesticides in Okagwe and Ihe-Nta, Abia State, Nigeria

ABSTRACT

Aim: To evaluate the levels of some kidney and liver function markers in humans exposed to pesticides in Okagwe and Ihe-Nta, Abia State, Nigeria.
Study design: A cross-sectional study.
Place and Duration of Study: Abia-ADP, Ohafia Zone and Department of Biochemistry, University of Port Harcourt, Nigeria, between August 2018 and September 2021.
Methodology: A total of 160 human subjects participated in this study. They consisted of 80 pesticides exposed agricultural workers in open field and 80 non-exposed volunteers, who served as control subjects. All the participants gave written informed consent, while the Ministry of Agriculture, Abia State, Nigeria gave Ethical Approval for this study. Venous blood samples were collected from all participants and dispensed into plain sample containers. The blood samples were allowed to clot and were centrifuged to obtain serum samples, which were used to determine kidney function tests: urea, creatinine, cystatin C, and kidney injury molecule -1 and liver function tests: aspartate amino transaminase (AST), alanine amino transaminase (ALT), total and direct bilirubin levels and total protein.
Results: Results showed that there were statistically significant decreases ($p<.05$) in serum total protein 39.60±5.58 (g/dl), total bilirubin 15.62 ± 3.71 (umol/l) in pesticide exposed agricultural workers compared to control. There were also statistically significant increases in AST 19.59 ± 2.45 (IU/L), ALT 70.34 ± 4.89 (IU/L), KIM-1 89.79 ± 8.85 (pg/ml), cystatin C 606.47±76.89 (mg/dl), urea 5.91± 0.60 (mmol/l), creatinine 65.31±5.56 (mg/dl) in pesticides exposed agriculture workers compared to control.
Conclusion: From the results, we conclude that there was liver and kidney damage as a result of this exposure to pesticide as shown by the significant increase in AST, ALT, cystatin C, urea and creatinine.

Keywords: kidney and liver function markers, pesticides, Okagwe and Ihe-Nta, Abia State, Nigeria

1. INTRODUCTION

For thousands of years, man has been investing so much efforts and available resources at eliminating weeds, pests and insects from plant crops for improve agricultural yield. This gave rise to emergence of pesticides. According to the Food and Agricultural Organization of the United Nations, pesticides are those substances or mixture intended for preventing, destroying or controlling any pest, including vectors of disease, weeds, animals, causing harm to the production of crops which may be administered to animals for the control of insects, arachnids or other pest [1]. In the world today, approximately 1,500 active ingredient have been registered as pesticides and formulators mix these compounds with one or more of some 900 inert materials to create approximately 50,000 commercial pesticide preparations registered for use [2].

It is important to note that occupational exposure to these pesticides occur from skin absorption and inhalations. The exposure of pesticides mainly occurs during the mixing and loading of the equipment, also in the spraying of these insecticides and improper handling [3]. The farmers who use these pesticides have only little or no access to information about proper use or the precautions needed when handling pesticides. Therefore, they often do not use even the simplest hygienic and protective measure [4]. Biological monitoring of pesticides exposure can be carried out by determining intact compounds of their metabolites in the blood, serum, plasma or urine [5].

It has been observed that in our environment, most of the chemicals exist as mixtures and their toxicity is mainly attributed to their interactions. However, assessment of the potential health hazard of chemical mixtures is difficult. These chemicals including pesticides is increasingly becoming a challenging toxicological problem, and a subject of major current concern to both the scientific and regulatory communities. Fukuyama et al. [6] observed that organ specific and systemic oxidative stress may play an important role in the toxicity of various pesticides including, organochlorine, as such continuous review of its pathological effect on the liver and kidneys of humans exposed to pesticides becomes necessary. There is still scarcity of reports on the toxic effect of pesticides on the human handlers/agricultural workers generally and particularly in Nigeria. This study is therefore designed to bridge this gap in knowledge. Therefore, the aim of this study was to evaluate the levels of some kidney and liver function markers in humans exposed to pesticides in Okagwe and Ihe-Nta, Abia State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This research was carried out at Okagwe and Ihe-nta in Ohafia Local Government Area of Abia State, Nigeria. The work was done with the assistance of the agricultural extension workers who provided technical advice to the local farmers. Ohafia is located at longitude 7.5247∈ and latitude 5.4309N. People in this area are predominantly farmers, and they are involved in both subsistence and commercial types of farming. Land tenure system is commonly practiced there. Ohafia is one of the local Government Area (LGA) in Abia State, Nigeria. It is an Igbo speaking region. It has its ancestral capital at Achi-chi Elu and has its LGA headquarters at Ebem Ohafia, Abia State, Nigeria. The current estimated population of Ohafia LGA is put at 234,700 inhabitants. People in this area are mostly Christians while some are traditionalists.

2.2 Sample Size Determination

The sample size was determined according to the method of Naing et al. [7].

2.3 Study Population

Human subjects were investigated in this study. This comprised of one hundred and sixty (160) subjects, eighty (80) of these subjects comprised of both Agricultural extension workers and adult farmers who are living in the same study area exposed to pesticides, and eighty (80) non-farmers who are living in different locality not exposed to pesticides as control subjects.

2.4 Selection Criteria for Subjects

2.4.1 Inclusion Criteria

The human subjects were between the age ranges of 25-50years. They must have been using pesticides in farming. They must have been residents in the same locality of the farm. Those not having any history of chronic disease like Diabetes Mellitus and AIDS.

2.4.2 Exclusion Criteria

Those subjects that had history of chronic disease like Diabetes Mellitus and AIDS and those on steroids.

2.5 Sample Collection

With the use of a syringe, 10ml of blood sample was collected from the anti-cubital vein of the subjects. The blood samples were dispensed into and plain containers. The blood samples were allowed to clot and were centrifuged to obtain serum samples, which were used to determine liver and kidney function markers.

2.6 Laboratory Procedures

2.6.1 Method of Determination of kidney function tests

2.6.1.1 Estimation of serum cystatin C (CYS-C) ELISA Kit
This assay employs the quantitative enzyme immunoassay technique (double–antibody sandwich) to assay cystatin c (cys-c) in serum or blood plasma.

2.6.1.2 Estimation of Kidney Injury Molecule -1 (KIM-1) ELISA Kit
The assay employs the quantitative enzyme immunoassay technique (double-antibody sandwich) to assay kidney injury molecule 1(kim-1) in serum, or blood plasma.

2.6.1.3 Estimation of serum urea concentration
This method is based on urease, urea will undergo hydrolysis to form NH_3 and Co_2. The ammonia will then react with phenol and hypochlorite to form indophenols (blue) which is measured spectrophotometrically at 546nm.
Urea $+H_20$ urease $2NH_3 + Co_2$
NH_3 + Hypochlorite + Phenol -------- indopenol (blue)

2.6.1.4 Estimation of serum creatinine
This method is based on the jaffe reaction; in this reaction creatinine reacts with picrate ion formed in alkaline medium to develop a red-orange color. The color produced from the sample is then compared in a colorimeter at wave length of 505nm with that produced by a known amount of creatinine under the same condition.

2.6.2 Method of Determination of liver function tests

2.6.2.1 Estimation of serum total protein
Serum Total protein (The Biuret Method) principle, Cu in alkaline solution reacts with the peptide bonds in protein, producing a violet colour which is proportional to the amount of protein present. After the mixing and incubating at 37^0c for 10 minutes then read at 540nm, using distilled water to zero the instruments.

2.6.2.2 Estimation of serum bilirubin (Jendrassik and Grof, [8])
Serum Bilirubin by Malloy and Evelyn, Bilirubin reacts with diazotized sulphalinic acid to form a purple colour compound, azobilirubin. Conjugated bilirubin reacts in an aqueous solution (direct reaction) whereas unconjugated bilirubin requires an accelerator or solubilizer such as alcohol (indirect reaction). After incubating for 5 minutes, it will be read at 540nm.

2.6.2.3 Estimation of serum glutamic pyruvic transaminase
Serum Glutamic Pyruvic Transaminase by Reitman and Frankel [9] principle. The pyruvate produced by the transamination activities of Gamma Pyruvic transaminase (GPT) react with 2,4 dinitrophenyl hydrazine (DNP to give a brown coloured hydrazine which is measured calorimetrically at 510nm.

2.6.2.4 Estimation of serum aspartate amino transferase

Aspartate amino transferase (AST) Principle: Oxaloacetate reacts with AST which decarboxylase it spontaneously to pyruvate which is measured by hydrazine formation. The brown colour is measured calorimetrically at 510nm.

2.6.2.5 Estimation of serum albumin
Albumin reacts with dye-Bromocresol green which has a specific affinity for it in acidic medium to produce a coloured complex which is measured colourimentrically at 540nm. The intensity of this colour produced is proportional to the concentration of albumin in the sample.

2.7 Statistical Analysis
Statistical analysis of data was done using IBM SPSS version 23 computer software. Descriptive statistics and standard deviations were used to summarize the characteristics for the study population, and values presented as (mean±standard deviation). Student's paired-sample t-test were used to compare the significant difference in the levels of exposure between farmers and control group. Results were presented in tables and *P<.05* were considered statistically significant.

3. RESULTS AND DISCUSSION

Table 1: Mean±SD values of serum liver function test of the study population exposed to pesticides in Okagwe.

Groups/ Parameters	Humans Exposed Mean±SD	Humans Control Mean±SD	P-value	Remark
Total Bilirubin (umol/l)	19.9± 3.29	12.12±1.24	<0.0001	S
Direct Bilirubin (umol/l)	22.15±1.95	21.83±1.63	0.066	NS
SGOT/AST (iu/l)	20.79±2.07	13.75±2.25	<0.0001	S
SGPT/ALT (iu/l)	69.93±5.93	49.33±3.60	<0.0001	S
Total Protein(g/dl)	61.50±5.51	72.67±7.25	<0.0001	S

Key: S-significant, NS –non-significant.

Table 2: Mean±SD Values of Serum liver function test of the study population exposed to pesticides in Ihe-Nta.

Groups/ Parameters	Humans Exposed Mean±SD	Humans Control Mean±SD	P-value	Remark
Total Bilirubin (umol/l)	25.60±2.27	19.02±1.36	<0.0001	S
Direct Bilirubin (umol/l)	23.55±1.92	23.31±1.63	0.074	NS
SGOT/AST (iu/l)	18.16±2.14	11.98±1.96	<0.0001	S
SGPT/ALT (iu/l)	70.73±3.61	49.96±3.85	<0.0001	S
Total Protein(g/dl)	58.66±5.10	72.50±5.25	<0.0001	S

Key: S-significant, NS –non-significant.

Table 3: Mean±SD Values of serum liver function test of the study population exposed to pesticides in Okagwe and Ihe-Nta.

Groups/ Parameters	Humans Exposed Mean±SD	Humans Control Mean±SD	P-value	Remark
Total Bilirubin (umol/l)	15.62±3.71	22.71±3.89	<0.0001	S
Direct Bilirubin (umol/l)	22.66±2.04	22.81±2.06	0.392	NS
SGOT/AST (iu/l)	19.45±2.45	12.87±2.28	<0.0001	S
SGPT/ALT (iu/l)	70.34±4.89	49.72±4.01	<0.0001	S
Total Protein(g/dl)	39.60±5.58	79.38±	<0.0001	S

Key: S-significant, NS –non-significant.

Table 4: Mean±SD values of serum kidney function test of the study population exposed to pesticides in Okagwe.

Groups/ Parameters	Humans Exposed Mean±SD	Humans Control Mean±SD	P-value	Remark
KIM-1	86.5±8.20	40.0±3.05	<0.0001	S
Cystatin C	586.6±61.72	386.4±36.0	<0.0001	S
Urea(mmol/L)	5.94±0.75	2.81±0.47	<0.0001	S
Creatinine (mg/Dl)	66.0±6.20	64.6± 5.92	0.035	S

Key: S-significant, NS –non-significant.

Table 5: Mean±SD Values of Serum Kidney Function Test of the Study Population Exposed to Pesticides in Ihe-Nta.

Groups/ Parameters	Humans Exposed Mean±SD	Humans Control Mean±SD	P-value	Remark
KIM-1	93.2±8.15	43.2±5.23	<0.0001	S
Cystatin C	629.2±84.38	414.4±30.8	<0.0001	S
Urea(mmol/L)	5.97±0.40	2.84±0.53	<0.0001	S
Creatinine (mg/dl)	69.6±3.66	65.5±6.01	<0.0001	S

Key: S-significant, NS –non-significant.

Table 6: Mean±SD Values of Serum Kidney Function Test of the Study Population Exposed to Pesticides in Okagwe and Ihe-Nta.

Groups/ Parameters	Humans Exposed Mean±SD	Human Control Mean±SD	P-value	Remark
KIM-1	89.7±8.85	41.6±5.40	<0.0001	S
Cystatin C	606.4±76.8	400.2±47.5	<0.0001	S
Urea(mmol/l)	5.91±0.60	2.79±0.51	<0.0001	S
Creatinine (mg/dl)	65.3±5.56	33.6± 5.73	<0.0001	S

Key: S-significant, NS –non-significant.

The total protein measures the total amount of albumin and globulin in the body and in the present study, a statistical significant decrease (*P<.05)* in serum total protein was observed as can be seen in table 1, 2 and 3, and this observation can be attributed to the damaging effects of pesticide on liver cells as confirmed by the increase in the serum AST and ALT, also observed in this study. This finding is in line with Yang and Chen, [10[, who reported that the reduction in plasma protein in animals treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver, in addition, that decrease in blood protein may be due to loss of protein by either reduce protein synthesis or increase proteolytic activity or degradation. The above finding is also in agreement with Borges et al. [11], who says that a decline in total protein was observed in all farm workers exposed to pesticide, also stated that ALT and AST may increase due to cellular damage in the liver and that high level of these enzymes in serum are usually indicative of disease and necrosis in the liver of animal. However, the present study did not agree with the finding of Magned, [12], who stated that the mean levels of total protein were significantly increased in all age groups of workers exposed compared to controls, rather this finding agree with Bomhard et al.[13], who reported that pesticides exposure decrease

protein biosynthesis in the liver, and that lack of a significant alteration in protein level in his study might be due to a high intake of a non-vegetarian diet.
The statistical significant increase in serum total bilirubin level in this study (Tables 1, 2 and 3), could be attributed to increase in breakdown of erythrocytes due to action of pesticide activity, this finding is in line with Ahmed et al. [14], who says that total and direct bilirubin level was found in higher levels in almost all the persons exposed to pesticides. He went further to say that a prolonged exposure to pesticides, disturbed the normal red cell metabolism affecting the hepatic dysfunction and therefore the level of bilirubin in the blood is increased causing hyperbilirubinemia which may be due to the production of more bilirubin than the normal liver can excrete. However, this study did not agree with his finding on direct bilirubin. In this study there is a slight decrease in direct bilirubin level as compared to the control, this decrease could be attributed to the inability of the liver to conjugate due to the damage of the hepatocyte by action of the pesticides in question.
In table 4, 5 and 6 of the present study, a significantly increase levels were observed in serum Kim-1, Cystain C, urea and creatinine, which is a sign of abnormal renal function and this could be as a result of exposure to pesticide which could lead to lipid peroxidation and accumulation melondialdehyde (MDA). This findings is in line with Coca et al. [15], who says that plasma cystatin C and serum creatinine were similarly expressed in rats exposed to Roundup formulation at 248.4mg/kg body weight of glyphosate concentration, also that higher level of lipid peroxidation observed in his study could also be attributed to the depletion of antioxidant defense in the kidney of the rats exposed to roundup herbicide at the increasing concentration of glyphosate. This study also agree with Jamal et al. [16], who reported significantly increased ALT, AST, urea and creatinine found in pesticide sprayers of mango plantations as compared with the control group indicate liver damage following high exposure to pesticide. Moreover, that a decrease in serum acetyl cholinesterase activity in occupationally exposed pesticides sprayers indicate a high degree of pesticides absorption.

4. CONCLUSION

From the results, we conclude that there was liver and kidney damage as a result of this exposure to pesticide as shown by the significant increase in AST, ALT, cystatin C, urea and creatinine.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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