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PESTICIDE RESIDUES AND HEPATITIS RISK

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Abstract: Serum samples of patients presenting with hepatitis and controls were compared with the view of determining the association between exposure to pesticide residues and hepatitis. Samples were obtained from fifty patients presenting with hepatitis in Federal Medical Centre Annex D/gari, and fifty control samples were also taken. Extraction of pesticide residues was done following the laid down protocol, and analysed using GC-MS model 6890N Agilent Technology coupled with micro electron capture detector. The results obtained revealed that 45 (90%) of the case population have no identifiable risk factor for hepatitis. The mean concentration of p,p[1] DDT, o,p DDT, p,p[1] DDE, o,p DDE, γ-HCH, aldrin and chlorpyrifos 0.088µg/L, 0.091µg/L, 0.080µg/L, 0.099µg/L, 0.073µg/L, 0.031µg/L and 0.088µg/L respectively were detected at elevated levels in case population. This study confirmed exposure to pesticide residues among the studied population and revealed significantly higher levels ($p \leq 0.05$) in case population than controls. Thereby, suggesting that exposure to pesticide residues may be a significant risk factor for hepatitis.

Keywords: Organochlorine, organophosphorus, cancer, DDT, controls.

INTRODUCTION

Pesticides are a diverse group of chemicals used to kill or control pests, including plants, molds, microorganisms and insects. Pesticides are widely used in agricultural, and commercial and residential settings, making exposure to the general population ubiquitous. Humans are exposed to pesticides via dermal (skin) contact, ingestion, and inhalation (CDC, 2009). Occupational exposure to pesticides may also occur among individuals employed in agricultural activities, pesticide manufacturing, pesticide application, and forestry. Again, family members could be exposed if pesticides are introduced into the home (e.g., on clothing). Non-occupational exposure can also occur through residential use or dietary ingestion from contaminated food and drinking water (Alavanja *et al*., 2004; Franklin and Worgan, 2005). Residential proximity to farmlands where pesticides are applied indiscriminately is another important source of ambient environmental exposure, where pesticides applied from the air and ground may drift from intended sites (Rull and Ritz, 2003; Deziet *et al*., 2015).

Pesticides are broadly known to exert adverse toxic effects to humans following high-dose acute exposure; however, knowledge about chronic low-dose adverse effects to specific pesticides is more limited. Although the majority of pesticides currently registered for use in Nigeria, are neither overtly genotoxic nor carcinogenic in rodent studies, human cancer possibly resulting from

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exposures is an area of public concern and growing scientific interest. Several years after their introduction into the market, many pesticides have become increasingly suspected of carcinogenic activity. Yet, relatively few of these compounds have been labeled as carcinogens or as probably (IARC group 2A) or possibly carcinogenic (IARC group 2B) to humans, none has been classified as carcinogenic without reservations (IARC group 1). Again, those compounds considered as possible or (especially) probable carcinogens may be thought of as being most likely to actually cause cancer in humans (IARC., 2016).

Pesticides are usually metabolized in the liver and are hypothesized to contribute to liver carcinogenesis through mechanisms of cell adhesion alterations, oxidative stress, genotoxicity, tumor promotion, immunotoxicity, and hormonal action (Dich *et al.*, 2007; Gomas *et al*., 2008; Jin *et al.*, 2014; Jin *et al*., 2014b). Experimental studies have shown that exposure to dichlorodiphenyltrichloroethane (DDT), and it's metabolite dichlorodiphenyldichloroethylene (DDE) and many other organochlorine insecticides widely used in agriculture and other household purposes have led to the development of hepatocellular carcinoma (HCC) and other liver tumors in rodents (Ross *et al.*, 2013; Turusov *et al*., 2013; Cohn *et al*., 2017).

Primary liver cancer has been said to be the sixth most common cancer in the world and the second leading cause of cancer-related death (Ferlay *et al.*, 2013). Approximately 70–85% of primary liver cancer cases are hepatocellular carcinoma (Jemal *et al*., 2011). The second most common histology is intrahepatic cholangiocarcinoma (McGlynn *et al*., 2015). Over 80% of HCC cases occur in East Asia and sub-Saharan Africa (Carr, 2010). Most of the HCC risk factors contribute to carcinogenesis by promoting the formation and progression of cirrhosis (El-Sarag and Kawal, 2014). In parts of Asia and sub-Saharan Africa, predominant risk factors include chronic hepatitis B virus (HBV) infection and exposure to aflatoxin, a mycotoxin produced by the *Aspergillus* fungus forming on foods in damp conditions (Yu and Yuan, 2004). Other risk factors include obesity, diabetes, non-alcoholic fatty liver disease (and non-alcoholic steatohepatitis), and cigarette smoking (Bravi *et al*., 2013; Duan *et al*., 2014). However, between 15- 50% of HCC cases have no established risk factors (Cohn *et al*., 2017).

Hepatitis is a significant and growing public health burden and a substantial number of cases are unexplained by known risk factors. A growing body of literature has examined pesticide exposure as a potential environmental factor related to hepatitis. However, to date, the literature has not been very precise on it.

In order to determine the unexplained risk factors of hepatitis, there has been continued interest in the role of pesticide residues especially the organochlorines and other persistent environmental pollutants (Calle *et al.*, 2012). Moreover, the rising incidence of liver cancer has aligned with the chronological patterns of release of persistent organic compounds in the environment. Moreover, the observation that the rising incidence of hepatitis has aligned with the chronological patterns of release of pesticide residues in the environment and attribution of a small fraction of hepatitis to known risk factors make it reasonable to investigate whether chemicals that persist in the environment are potential risk factors for hepatitis. This hypothesis that pesticide residues might increase the risk of hepatitis is based on the carcinogenic and the weak hormonal (oestrogenic and anti-oestrogenic) effects of many pesticides and their metabolites (Kleanthi and Andreas, 2009). Although, many studies have investigated the relationship between pesticides and liver cancers, the results have remained inconclusive (Brody and Rudel, 2003; Schottemfeld and Fraumeni, 2006; Kleanthi and Andreas, 2009; Calle *et al.*, 2012).

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Although most environmentally persistent pesticides importation, sales and use have been banned in Nigeria, there are evidences of continued usage and presence in the ecosystem. For example, Adeyemi *et al*. (2011) evaluated concentration of OCP residues in water samples of Lagos Lagoon and reported that the mean concentration of organochlorine pesticides detected were higher than the European Community allowable residual limit for individual OCPs in drinking water in 37.3% of samples analyzed. Also, Sosan *et al.* (2015) examined DDT and HCH residues in foodstuff sold in markets in Ile-Ife, southwestern Nigeria and concluded that organochlorine pesticides are still being used to the extent that they were detected in cowpea grains and dried yam chips in concentration above the European Union- Maximum Residue Limits (EU-MRLs). Again, Osesua *et al*. (2019) in their study of pesticide residues in fish and water from Dukku River in Birnin Kebbi reported that the banned pesticides are still used indiscriminately in agriculture. These recent studies showed that Nigerians are still greatly exposed to OCPs in spite of the reported association between liver cancer and pesticides exposure. Despite several studies on relationship between pesticides and liver cancer in developed countries, the continuing exposure of Nigerians to these chemicals and the increased rate of liver related diseases incidences in Nigeria, there is a dearth of data on the relationship between pesticides usage and hepatitis incidence in the country. Thus, this present study was undertaken to comprehensively evaluate the residue levels of commonly used pesticides in the serum samples of diagnosed hepatitis patients in Federal Medical Centre (FMC Annex), Darkin Gari and control subjects. This will provide baseline information on the possible association between these pesticide residues and hepatitis incidence in the state.

MATERIALS AND METHODS

The Study Design

Dakingari town is the headquarters of Suru Local Government Area in Kebbi State, Northwest Nigeria. About 80 percent of the nearly 200,000 people in this town are predominantly farmers. The anxiety amongst the farmers to increase crop yield and improve productivity necessitate total reliance on agro-chemicals especially pesticides. Blood samples (n=50) were collected from patients diagnosed with hepatitis in Federal Medical Centre (Annex), Dakingari between January 2017 and December 2019. Samples of blood (n=50) were also collected from inhabitants of the area with no history of hepatitis who are mostly civil servants and traders and used as control. Written consent was obtained from each of the participants and those who were unwilling to give consent were excluded from the study and the protocol was approved by Ethical and Research Committee of Federal Medical Centre, Birnin Kebbi, Nigeria.

Data were collected from cases and controls with a pre-designed questionnaire that sought information on the medical history, social lifestyle and other risk factors that could predisposed a patient to having hepatitis.

Sample Collection

Blood samples (5mL) were collected in residue free heparinised glass vials with the help of sterilized syringe after proper cleansing of the venipuncture site with a swab soaked in methylated spirit. Blood samples of control were transported in dry ice to the laboratory, while blood samples of patients presenting with hepatitis were collected *in situ* in the hospital. All samples were stored at – 20°C until they were analyzed. Samples were analyzed using GC-MS model 6890N Agilent Tech. coupled with ECD.

Solvents and glassware

All the solvents, including acetone, diethyl ether and hexane (HPLC grade), used for the analysis were purchased from E-Merck. Organic solvents were glass distilled and checked for any pesticide contamination. All glassware were washed with detergent, rinsed with water,

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dipped in chromic acid for 24 hrs and finally rinsed with distilled water and then hexane.

Chemicals

Pesticide reference standards were purchased from Sigma Aldrich Chemicals Germany through Bristol Scientific Co. Nigeria.

Sample Extraction and Clean up

Extraction

Extraction was based on the method followed by Agarwal *et al*. (2016) as modified by Osesua, *et al*. (2018). Blood (5 mL) was diluted with 25 mL distilled water and a 2 mL of saturated brine solution was added, then it was transferred to a 125 mL capacity separatory funnel. It was extracted with hexane: acetone (1:1) (20 mL) (thrice), by shaking the separatory funnel vigorously for 2 - 3 min, thereby releasing the pressure intermittently. Consequently, the layers were allowed to separate. The three combined extracts were passed through anhydrous sodium sulfate and concentrated to about 1 – 2 mL using a rotary vacuum evaporator.

Clean up

Clean up was done using USEPA method 3620B (2015), (Florisil clean up by column chromatography). Florisil was activated at 130°C overnight and cooled in a dessicator before use. The weight of florisil taken was predetermined by calibration using lauric acid and 1 g of florisil was packed in the 20 cm length and 12 mm i.d glass chromatographic column. Anhydrous sodium sulfate was added to the top of the florisil column (0.5 cm) and the column was pre-eluted with hexane, and was then discarded. The extract was transferred to the column and eluted with hexane (10 mL), 6% diethyl ether in hexane (10 mL), 15% diethyl ether in hexane (10 mL), 50% diethyl ether in hexane (10 mL) and finally with diethyl ether (10 mL). As a result, eluent was collected and evaporated to dryness. The final samples were prepared in 2 mL hexane (HPLC grade) and were then analysed

Sample Analysis

Calibration of GC-MS system: GC system was calibrated using external standard technique.

Stock Standard Solution (1000 mg/l)

Stock solution was prepared by weighing appropriate amounts of active ingredients in a brown bottle with a teflon-lined screw cap and dissolving the weighed standard in HPLC grade hexane. Stock standard solution was used to prepare primary dilution standards.

Quantification of the Pesticides

To get the final concentration of each analyte or total analytes, the formular below was used

$$\text{Final concentration} = \frac{\text{GC result of analyte from chromatography x dilution factor}}{\text{Volume of Sample taken for extraction}}$$

where;

$$\text{Dilution factor} = \frac{\text{Final Volume of Extract}}{\text{Weight of dried clean} - \text{up extract}}$$

The percentage recovery was evaluated from the relationship:

$$\%\text{R} = \frac{A' - A}{B}$$

where A' is the amount of a pesticide in the spiked sample, A is the amount of pesticide in the unspiked sample and B is the amount of pesticide used for spiking. Percentage recovery was found to be between 92-100% indicating that the analytical procedure was effective

RESULTS AND DISCUSSION

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Table 1: Mean Conc. (µg/L) of Pesticide Residues in the Blood Samples of Patients Presenting with Hepatitis (Case)

Pesticide	Mean Conc.	Range	%	No of Samples > MRL
p,p^1 DDT	0.088±0.007	0.005-0.167	100	21
o,p DDT	0.091±0.012	0.003-0.190	100	22
p,p^1 DDE	0.080±0.011	0.003-0.177	98	25
o,p DDE	0.099±0.017	0.004-0.198	100	28
α-HCH	0.003±0.001	ND-0.007	12	0
γ-HCH	0.073±0.008	ND- 0.105	39	9
δ-HCH	0.003±0.002	ND-0.005	09	0
Aldrin	0.031±0.015	0.001-0.113	51	16
Endosulfan	0.024±0.009	0.002-0.110	38	09
Heptachlor	0.019±0.005	ND-0.112	21	05
Chlorpyrifos	0.088±0.033	0.021-0.199	98	18
Malathion	0.021±0.009	0.002-0.108	10	2
Parathion	0.030±0.007	0.001-0.119	10	3
Diclorvos	0.009±0.003	ND-0.017	03	0
Phosphamidon	0.007±0.002	ND-0.012	02	0

Table 2: Mean Conc. (µg/L) of Pesticide Residues in the Blood Samples of Control

Pesticide	Mean Conc.	Range	%	No of Samples > MRL
p,p^1 DDT	0.009±0.002	0.001-0.102	33	2
o,p^1 DDT	0.011±0.005	0.001-0.111	35	3
p,p^1 DDE	0.012±0.009	0.001-0.121	37	3
o,p^1 DDE	0.013±0.007	0.002-0.138	31	5
α-HCH	0.002±0.001	ND-0.004	3	0
γ-HCH	0.013±0.007	ND- 0.059	12	1
δ-HCH	0.002±0.001	ND-0.005	2	0
Aldrin	0.005±0.002	0.001-0.090	5	1
Endosulfan	0.007±0.002	0.001-0.044	3	1
Heptachlor	0.007±0.003	ND-0.014	4	1
Chlorpyrifos	0.018±0.009	0.005-0.109	27	05
Malathion	0.005±0.002	0.001-0.010	09	0
Parathion	0.006±0.001	0.001-0.012	7	0
Diclorvos	0.003±0.001	ND-0.007	1	0
Phosphamidon	0.004±0.002	ND-0.010	2	0

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Table 3: Social/Demographic Characteristics of Cases and Control

Characteristics	Cases (%)	Control (%)
Age Group (Years)		
< 40	37	41
≥ 40	63	59
Sex		
Male	87	79
Female	13	21
Occupation		
Artisan/Trading	2	82
Farming	95	2
Civil Servants	3	16
Medical History		
Blood Transfusion	0	3
Social History		
Alcohol Consumption	0	1
Hard Drugs	0	0

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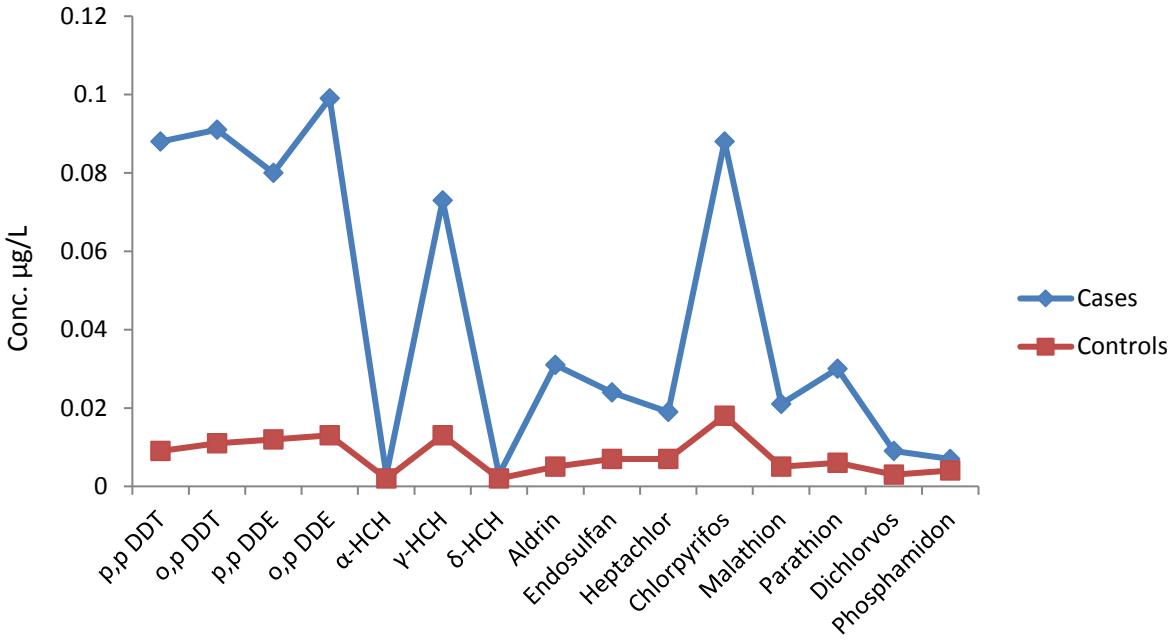

Figure 1: Mean Comparison between Pesticide Residues in Cases and Controls

DISCUSSION

The mean age of participants both cases and controls was 43.7 years with a range of 30-65 years. The mean body mass index (BMI) was 28.16 kg/m. Of the 95% case volunteers who are farmers, 32 reported history of spraying pesticides in their fields without using any protective measures. Among the case participants, 28 were addicted to tobacco chewing even during working hours.

Table 1 shows that serum samples collected from 50 case patients were found to be contaminated in various degrees. 100% of the samples were contaminated with p,p^1 DDT, o,p DDT and o,p DDE, 98% were contaminated with p,p^1 DDE. Of these values the number of samples where pesticide residues concentrations were greater than the maximum residues levels (MRL) are 21, 22, 25 and 28 respectively. Fifty-one percent (51%) of the case samples were contaminated with aldrin having 16 of those samples in concentration above MRL. γ-HCH was detected in 39% of case samples with 9 above MRL, endosulfan detected in 38% of case samples with 9 above the MRL. Chlorpyrifos one of the organophosphorus analysed was detected in 98% of case samples with 18 above MRL. α–HCH, δ-HCH, dichlorvos and Phosphamidon were however detected in the case samples with concentrations below the MRL.

Table 2 on the other hand shows the residues concentration in the serum samples of the controls. Perusal of the table revealed similar contamination pattern but with lower concentration levels, lower prevalence and lesser samples detected having concentrations above MRL.

The presence of p,p^1 DDE and o,p DDE alongside the parent DDT may indicate the long-term persistence of

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DDT metabolites in the human body. Further, metabolism of DDT in the human body leads to conversion into DDE and DDD metabolites. Though DDT use is banned in many countries including Nigeria, its use still continued illegally due to lack of suitable alternative and low cost (WHO, 2016; Osesua *et al.*, 2018). The residue levels of DDT and DDE in this work indicated that highest mean concentration of total DDT was observed in the age group of ≥40 years with 68.9% of detection frequency. A strong and positive correlation of DDT residues with age of participants was noticed (r=0.509). Mann–Whitney U-test results indicated statistically significant differences for total DDT values in all the age groups ($p<0.05$). Increased residue levels of DDT with the age of human were also reported earlier (Koutros *et al*., 2015). International Agency for Research on Cancer (IARC) has classified DDT and its metabolite DDE as a possible human carcinogen (Group 2B) (Ferlay *et al*., 2013).

HCH is known for its broad spectrum insecticidal properties. It usually undergoes degradation into different metabolites. Of the three metabolites of HCH detected in this work, the γ-HCH mean concentration 0.073µg/L was found to have the highest concentration and most prevalent. This could be due to the fact that the γ-HCH is a more potent insecticide and is most commonly used. 9 case samples were detected at concentration above the MRL, while α-HCH and δ-HCH having mean concentration 0.003µg/L were found to be below the MRL in all the case samples. γ-HCH was found to be toxic to the kidney and liver after administration orally, dermally or by inhalation in short-term and long-term studies of toxicity and reproductive toxicity in rats (Wasserman *et al.*, 2016). It was observed to induce hepatocellular hypertrophy in a number of studies in mice, rats and rabbits and was known to increase incidence of adenomas and carcinomas of the liver in agouti and pseudoagouti mice (Hudson *et al*., 2014). IARC classified γ-HCH as a possible human carcinogen (Group 2B).

Aldrin is a broad spectrum, contact organochlorine pesticides that has been widely used in the past to control soil insects such as termites and ants. Its use has been severely restricted in many countries since the 1970s, but it is known to extremely persistent in the environment and in humans and animals. Aldrin can easily metabolize to dieldrin, and was classified by IARC as probably carcinogenic to humans (Group 2A). For aldrin, there was sufficient evidence for cancer in experimental animals, but epidemiological data on aldrin were inadequate. However, since aldrin rapidly converts to dieldrin in the body, exposure to aldrin inevitably entails internal exposure to dieldrin (IARC. 2016). The mean concentration of aldrin in case samples was 0.031µg/L detected in 51% samples of which 16 were above the MRL.

Endosulfan was detected in 38% of the case samples and 9 of the samples have concentrations above the MRL. Endosulfan causes oxidative stress (Omurtag *et al*., 2008), which is implicated in its neurotoxic effects (Jia and Mizra, 2007a,b), damage to the adrenal gland (Dorval and Hontela, 2013; Dorval *et al*., 2013), and in cancer (Antherieu *et al*., 2017). The brain is particularly sensitive to oxidative damage from sublethal levels of endosulfan (Ballesteros *et al*., 2018). The primary systemic targets are the liver and kidney, but it also causes haematological and respiratory effects as a result of generalised effects on the central nervous system. However, endosulfan has caused mutagenic and genotoxic effects in human lymphocytes and liver hepatoblastoma cells (Jamil *et al*., 2014; Lajmanovich *et al*., 2015). Endosulfan has not been classified by the International Agency for Research on Cancer (IARC) as a carcinogen, and was described by the International Programme on Chemical Safety (IPCS) (2000) as not carcinogenic, but it is increasingly being

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described as a potential carcinogen in humans (Antherieu *et al*., 2017).

Case samples were found to have heptachlor residues in 21% of the analysed samples and 5 of the samples were detected at concentrations above the MRL. Heptachlor was classified by EPA as a probable human carcinogen, but recent classification by IARC placed heptachlor as possibly carcinogenic to humans. Heptachlor was found to be carcinogenic to mice at doses of 0.5 mg/kg body weight and above. It acts as a tumour promoter in mouse liver. It is unlikely that tumours in mouse liver are induced through a genotoxic mechanism. Therefore, non-neoplastic effects (histopathological liver changes, neurotoxicological effects, and immunotoxicological effects) were chosen as the basis for the derivation of a tolerable intake; they have been reported at about $1/20^{th}$ of the concentration at which carcinogenic effects were seen (Amita *et al*., 2013).

The mean concentration of malathion was 0.021µg/L in case samples with 2 detected above the MRL. Malathion was classified by EPA as probably carcinogenic to humans due to its ability to induce liver mass, foci and nodules increased with dosage (EPA, 2014; Hoshiya *et al*., 2013).

Chlorpyrifos (98%) of the case samples analysed, indicated regular and widespread exposure. Concentration of chlorpyrifos was very high as compared to that detected by Schafer *et al*. (2014), who reported that chlorpyrifos and parathion were the major organophosphorus residues found in blood at levels up to 4.5 times higher than what the U.S government deems acceptable. The reason for the very high concentration of these pesticides can be hypothesized in the fact that these compounds form a major component of pesticide formulation used in the study area.

Chlorpyrifos has a mean concentration of 0.088µg/L in case samples 18 of which were detected to be above the MRL, parathion has 3 samples above MRL, while dichlorvos and phosphamidon were detected below the MRL.

In this present study none of the participants (both cases and controls) had family history of hepatitis or liver cancer. Alcohol consumption and smoking as risk factors of liver cancer were poorly represented in this study among the case population in that none of them have taken alcohol before and about 1% of the controls (Table 3) had taken alcohol before or smoked before, though it is not impossible that they are secondary smokers. Again none of the participants have taken hard drugs like narcotics, stimulant drugs or any steroidal preparations. Moreover, about 3% of the control population had blood transfusion before, while none of the case population ever had blood transfusion. Since none of the case population ever had blood transfusion, alcohol and hard drugs and higher number of the control population (3%) had blood transfusion and (1%) used alcohol before, blood transfusion and alcohol intake cannot be adjudged to be the risk factors in the population studied.

From the result obtained on the known risk factors of hepatitis and liver cancer (medical history, alcohol intake, blood transfusion and drug abuse), this study established that most case population had no identifiable risk factors. It is therefore very imperative to reasonably conclude that the known risk factors for hepatitis and liver cancer in the studied population account for very few of the cases. This is in agreement with the reports that the known risk factors are thought to account for less than 50% of cases of hepatitis and cancer of the liver that actually occurred (Brody and Rudel, 2013). Thereby, corroborating the suggestion made by Calle *et al* (2012) that it is reasonable to investigate whether environmental chemicals that persists in the environment especially pesticide residues as potential risk factors for hepatitis and liver cancer.

Mean levels of all the residues (organochlorine and organophosphorus) pesticide compounds analysed were significantly higher in the hepatitis patients than the

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controls (Figure1). This suggests a positive association between these compounds and the risk of hepatitis. This positive association between DDT its metabolite DDE, aldrin, endosulfan and hepatitis has been reported by many earlier epidemiological studies (Hoyer *et al.,* 2010; Charlier *et al.,* 2013; Cohn *et al.,* 2017) but inconsistent with Romieu *et al.,* (2010) and Gammon *et al*. (2012) that suggested that DDT, DDE and endosulfan exposure cannot be linked to increased hepatitis or liver cancer risk. However, researchers from the California-based Public Health Institute and Mount Sinai School of Medicine in New York based on their findings from a five-decade study that DDT exposure was found to predict a four-fold increase in hepatitis and liver cancer risk (Cohn *et al*., 2015).

The result of significantly higher levels of γ-HCH and chlorpyrifos also revealed that the mean concentration of γ-HCH in case patients is about 10-fold that of controls while mean of chlorpyrifos in cases quadrupled that of controls. Aside the significant higher mean, residues of the two compounds were found more frequently in the hepatitis patients than controls. This finding agrees with the report of Mussalo-Rauhanaa *et al.* (2010) who found residues of γ-HCH, chlorpyrifos and heptachlor more frequently and higher in case patients than in controls. However, this recent study is not consistent with some other findings that found insignificant lower mean concentration of HCH in liver cancer patients (Zheng *et al.*, 2015; Li *et al*., 2016)

The inconsistent conclusion of several studies on the association of pesticide residues and hepatitis or liver cancer risk in relation to the present study can be attributed to several factors which include difference in sample size, use of different biological specimens (blood/adipose tissue), type of control population (free of tumor or benign tumor), possible individual variation in the rate of metabolism of pesticides and time of study.

The higher levels of the residues found in the older age group may be due to longer period of exposure. Highest levels found in farmers may be due to occupational exposure (Table 3). Farmers in their anxiety to control pest and improve yields have resulted to indiscriminate use of pesticide in virtually every phase of agricultural activities with little or no protection (Rubin *et al.,* 2016). Higher levels found among the civil servants and artisans that reside in city may be as result of non-occupational exposure due to excessive use of some persistent pesticides to preserve food consumed in the city. Based on gender, it was observed that the prevalence was higher in males than females of both populations as shown in Table 3, but residue levels did not show any statistically significant difference at $P \leq 0.05$.

CONCLUSION

The evidence to date concerning the association between pesticide residues (organochlorine and organophosphorus pesticides) exposure and risk of hepatitis or liver cancer is not entirely consistent. This study however found that exposure to some pesticides residues especially the highly persistent (DDT, DDE, γ-HCH, aldrin, and endosulfan) can increase the risk of hepatitis or liver cancer. In addition, this work has shown that exposure to environmental pollutants is a major risk factor of hepatitis because most of the hepatitis patients present no identifiable known factors of hepatitis. Notable is the fact that, although, most of the identified pesticide residues and metabolites have been banned in Nigeria, there is an indication that some of these pesticides are still in use as at the time of this study as occasioned by the high presence of both the parent compound and its metabolites in the blood samples.

Although, this study was limited by small sample size, the population selection methods were refined by age-matching controls to address the sample size limitation and increase the potencies of these findings. This study has provided for the first time information on the possible

association between hepatitis and pesticide residues and help in finding answer to the question of high prevalence of hepatitis amongst the local farmers in the study area and also provided background useful information on levels of these compounds in the studied population.

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