Variations in Cholinesterase activity in Oil Palm Plantation Workers Exposed to Pesticides

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ABSTRACT

Pesticides including organophosphates (OPs) and carbamates are used extensively in public health and agriculture to increase crop yields. The abuse of pesticides can have a serious impact on farmer's health. The objective of this study is to study changes in serum cholinesterase activity (BChE) as a biological marker of PO poisoning. Workers in a palm tree oil unit during periods of exposure and non-exposure to pesticides, as well as a control group were selected randomly. The clinical signs reported are those observed in the subjects exposed to organophosphates and anticholinesterase carbamates. The serum enzymatic activity (AES) of cholinesterase was quantified by spectrophotometry. The average serum cholinesterase level during the exposure and non-exposure period was $4225.64 \text{ U / l} \pm 791.10$ and 4766.47 ± 873.32 versus $6588.95 \text{ U / l} \pm 1303$, respectively, 42 the control level ($n = 43$) ($p < 0.001$). The average enzymatic activity of workers ($n = 19$) during non-exposure and exposure periods increased from (78.95%) to non-exposure (36.84%) (Data Not shown) with a significant difference ($p = 0.00750$). Inhibition of enzymatic activity greater than 40% was observed during the non-exposure phase in 21.05% of cases, and during the exposure period in 26.32% of cases, a bi-annual biological factor necessary for these workers.

INTRODUCTION

The use of pesticides is currently the main control strategy to guarantee the world's food supply. Most pesticides, however, are toxic to non-target species, including humans. In order to increase the harvest and obtain better yields, pesticides are used, and among these, the most used are organophosphates (OP) and carbamates. The widespread use of organophosphates pesticides throughout the world exposes to poisoning by these chemical agents, particularly in developing countries. The mechanism of toxicity is the inhibition of acetylcholinesterase leading to an accumulation of acetylcholine and the continuous stimulation of cholinergic receptors (Kwong, 2002). The activities of plasma cholinesterases or plasma (buthrylcholinesterase) and intra-erythrocyte are the indicators used to monitor workers exposed to organophosphates, indicating the effects of cumulative exposures of previous weeks or of acute overexposure (I.N.R.S, 2012). Intensive use of these products can lead to occupational diseases and poisoning (Karlsson, 2004; Sam and al 2008; Faria and al 2009). Exposure to pesticides in agriculture is mainly through dermal and inhalation contact (IPCS, 2004), and use of personal protective equipment (PPE) may help reduce exposure levels (IPCS, 2004; Blanco and al 2005). Studies to determine knowledge, attitudes and practices (PAC) on the use of pesticides have been conducted around the world to understand the working environments and conditions in which pesticides are handled and applied by agricultural workers (Ngowi and al 2002; Recena and al 2008; Ospina and al 2009, Karunamoorthi 2011, Ribeiro 2012). The harmful effects of pesticides are divided into two categories: acute toxicity and chronic effects. The acute toxicity of pesticides generally concerns the professionals in the phytosanitary field (manufacturers and users). The work of Wesseling and al. (2010) in Costa Rica showed a relationship between acute occupational poisoning with organophosphates, and suicidal ideation. Amar and al and dasgupta and al (2013, 2007) found a decrease in serum cholinesterase activity as a function of years of exposure to organophosphates pesticides. A survey conducted by Kamenan in 2000 in Abidjan among industrial pesticide handlers, showed that the proportion of workers affected by organophosphate poisoning was 80.7% (2000). Data collected on the health effects of pesticides on producers indicate that the health problems associated with the use of pesticides were recurrent. Of the 42 health centers surveyed, 922 cases of pesticide poisoning since 2002 have been identified. Each year, the national health laboratory records 800 cases of poisoning (5 to 10%) of cases due to pesticides.

The objective of this work is to determine changes in cholinesterase activity in order to assess the levels of poisoning of workers to

organophosphates pesticides and carbamates according to the period of exposure.

MATERIALS AND METHODS

This prospective study was conducted in Abidjan (Côte d'Ivoire) in a palm oil farming unit from January to October 2016.

Inclusion Criteria

The study included on one hand, cases of workers who had been in contact with phytosanitary products during the storage, preparation, spreading, disposal of residues and supervision of, and who observed a period of non-exposure varying between 1 and 3 months and, on the other hand, of persons having no regular contact with these products, and who came from the staff of the UFR values of normal biochemical parameters (blood glucose, urea, creatinine, transaminases, hemoglobin). Indeed, the biochemical parameters were assayed to eliminate anything that could contribute to a misinterpretation of the results of cholinesterase, since the cholinesterase activity is usually associated with liver damage, Anemia, a congenital deficiency, and an organophosphates poisoning in this case, there is first a decrease in serum activity and then a decrease in the globular activity. An increase in activity is observed in the case of nephrotic syndrome (Biommis 2012).

Informed consent for both cases and witnesses

For each person who meets our inclusion criteria, a fact sheet containing socio-demographic characteristics, professional information, pesticide application conditions, and clinical aspects has been completed. After information, informed consent, a promise of confidentiality and a return of information, a blood sample for the biological examinations was made. In this study, a worker is considered to be in the exposure period up to 5 days after pesticide use, and, within the non-exposure period after 1 month of non-use of pesticides.

Samples

The blood collected by venipuncture in fasting morning subjects was collected in dry Vacutainer tubes for the determination of urea, creatinine, transaminases and cholinesterase, tubes containing potassium oxalate and Fluoride for the determination of blood glucose and in tubes containing EDTA for hemoglobin (Hb). In cases: there were 2 withdrawals: 1st in exposure period and 2nd withdrawal in non-exposure period 1 to 3 months after a period of non-exposure. Samples were immediately conveyed to the laboratory in coolers containing carbo-ice. Tubes without anticoagulants and those containing

potassium oxalate and fluoride were centrifuged (centrifuge 5804) at 3000 rpm for 3 min for analysis.

Dosage

The parameters concerned are: blood glucose, urea, creatinine, transaminases, and hemoglobin which have been assayed as inclusion assay and cholinesterase. The hemoglobin assay was performed by the Sysmex KX-21N® hematology machine while all other parameters were analyzed by colorimetric methods with a HITACHI 902 auto-analyzer. The AES of serum cholinesterase was evaluated by spectrophotometry with kinetic reading. The dosage principle is as follows: Cholinesterase catalyzes the hydrolysis of butyrylthiocholine to butyrate and thiocholine. Thiocholine reacts with 5,5'-dithiobis-2-nitrobenzoic acid (D.T.N.B) to form nitro-2-mercapto-5-benzoate. The concentration of the product formed which is measured at 405 nm is proportional to the enzymatic activity of the cholinesterase present in the sample to be assayed. The normality range is between 4,640 -11,120 U / L for a reaction temperature of 37°C.

Statistical Analysis

Statistical analysis of the data was carried out using the Epi info 7 software. The relationship between serum cholinesterase and professional seniority was investigated using the Pearson correlation test. The relationship between clinical symptoms reported by workers and serum cholinesterase was investigated by the Student or Kruskal Wallis test, depending on the case. The study of the relationship between serum cholinesterase and the use of P.P.E was carried out by the Student or Kruskal Wallis test. The significance level was set at 95% (p <0.05). The average was compared with the Student test.

RESULTS

Socio-demographic and professional characteristics

Gender

The study population consisted of 19 males.

Age

The average age was 41 years ± 7.49 for people exposed and non-exposed, and 37.95 ± 11.99 for controls. The difference in age was not significant (p = 0.14).

Professional seniority

Average professional tenure was 15 ± 10.03 years with extremes of 2 to 37 years and had no correlation with the mean serum cholinesterase level at both the exposure and non-exposure periods.

Personal Protective Equipment (P.P.E)

For P.P.E use, 91.6% of workers were negatively correlated with the mean cholinesterase level during the exposure and non-exposure period.

Biological results

Mean values of cholinesterase in the study population

The mean values of cholinesterase in the study population are as follows:

Table 1: Mean serum cholinesterase levels in exposed and controlled subjects			
	exposure period	non-exposure period	Control subjects
Workforce	19	19	43
Minimum value (U/l)	2799	3114	4640
Maximum value (U/l)	6404	6300	11 120
Average value (U/l)	4225.65	4766	6 588.95
Median	4213	4999	6 275

The mean serum cholinesterase (BChE) level in exposed individuals was 4248.47 U / l ± 829.53 during the exposure phase and 4766 ± 873.32 during the non-exposure phase and that of the controls was 6588.95 U / l ± 1303.42 (Table 1). The average worker's BChE activity (n = 19) during exposure and non-exposure periods was significantly lower than for controls (n = 43). The comparison of these means with that of the controls showed a significant difference (p <0.001). Comparison of worker

averages during exposure and non-exposure periods gave a significant difference p = 0.01689.

Variation of inhibition of enzymatic activity during the non-exposure phase

Variations in the inhibition of enzymatic activity during the non-exposure phase were shown in the table below:

Table 2: Variation of the inhibition of the enzymatic activity during the non-exposure phase

Inhibition Degree	Frequency	Percentage
Strong	4	21.05%
Moderate	3	15.79%
Normal	12	63.16%
Total	19	100%

The inhibition of enzymatic activity during the non-exposure phase was normal in the majority of cases (63.16%).

Variation of the inhibition of the enzymatic activity during the exposure phase

Variations in the inhibition of enzymatic activity during the exposure phase were shown in the table below:

Table 3: Variation of the inhibition of the enzymatic activity during the exposure phase

Inhibition degree	Frequency	Percentage
Strong	5	26.32%
Moderate	10	52.63%
Normal	4	21.05%
Total	19	100.00%

The inhibition of enzymatic activity during the exposure phase was moderate in the majority of cases (52.63%).

Note:

Normal inhibition: < 30%

Moderate Inhibition: 30-40%

High inhibition: > 40%

Comparison average value of cholinesterase and reported symptoms

Comparison of average cholinesterase value before exposure and post-exposure with symptoms reported by workers gave a non-significant difference ($p = 0.5$)

Comparison average value of cholinesterase and occupational seniority

Comparison of the average value of cholinesterase before exposure and post-exposure with occupational seniority gave a non-significant difference ($p = 0.95$)

Comparison of Mean Value of Cholinesterase and Use of P.P.E

Comparison of the mean value of cholinesterase before exposure with exposure to occupational seniority gave a non-significant difference ($p = 0.43$)

DISCUSSION

Socio-demographic and occupational characteristics

Gender

All workers (100%) were male. The absence of women is due to the fact that the selected population was the one handling the plant protection products and in this undertaking this population is made up entirely of men. Our findings are consistent with those of Manda and al. (2010) who found 100% male subjects in their work.

Age

We found that the subjects in our series were adults with an average age of 41 years. These results are in agreement with those of Juliana and al. (2012) who found an average age of 41 years for people exposed and non-exposed. The difference in age was not significant ($p = 0.14$).

Use of EPI

With regard to the use of P.P.E, 91.6% of workers were employed. This result is different from some previous studies. Indeed, studies conducted by (Snelder and al 2008 Doumbia and Kwadjo, 2009) revealed that market gardeners protect themselves very little during treatment.

Organic Results

Mean values of cholinesterase in the study population

The average worker's BChE activity ($n = 19$) during exposure and non-exposure periods was significantly lower than for controls $n = 43$ ($P < 0.001$) (Table 1). The comparison of the averages of the workers during the exposure and non-exposure periods gave a significant difference $p = 0.01689$. Our results are different from those of (Juliana et al. 2012; Bulgaroni and al. 2012; Joshaghani and al. 2007) who found no significant difference between the average activities of the farmer group during non-exposure periods and exposure periods.

Variation of the inhibition of the enzymatic activity during both phases

Enzyme inhibition of butyrylcholinesterase was considered normal when the rate was $< 30\%$, moderate between 30% and 40% , and severe when $> 40\%$. Severe inhibition corresponds to a very great decrease in enzymatic activity, moderate inhibition corresponds to an average decrease in enzymatic activity, and normal inhibition to an enzymatic activity, the decrease of which is insignificant. Normal inhibition was (63.16%) and (21.05%) , respectively, during the non-exposure phase and during the exposure phase. Severe inhibition during the non-exposure and exposure phase was (21.05%) and (26.32%) , respectively. Moderate inhibition was (15.79%) and (52.63%) , respectively (Table 2-3). We observe that inhibition of enzymatic activity is higher in the majority of the population during the exposure phase (78.95%) than in the non-exposure phase (36.84%) with a significant difference ($P = 0.0075$). Our findings are consistent with Juliana et al. (2012) who found the majority of workers that had $\pm 30\%$ variation in enzymatic activity during the exposure period. For Fournier et al., (1970), decreases in blood activity of 50 to 70% of exposed individuals can be observed without associated clinical signs. Our results showed a reduction in serum cholinesterase activity of more than 40% during the exposure and non-exposure phase, respectively, in 5 and 4 workers, with an inhibition of 30% in 3 and 10 workers respectively (Table 2- 3).

According to the International Labor Organization (I.L.O.), a reduction of serum cholinesterase greater than 40% results in the withdrawal of the subject and his transfer to another post until the cholinesterase level (OIT, 2010).

Mean Cholinesterase Value Comparison

Comparison of the mean value of cholinesterase with clinical symptoms, professional age and use of PPE showed a non-significant difference. Our results agree with Juliana et al., (2012) who also found no significant correlation.

CONCLUSION

Organophosphates pesticides, which are widely used in agriculture, can be a source of intoxication among

workers. Consequently, good action (compliance with hygiene measures and instructions on the safety of use of pesticides) during their use is necessary to minimize the risk of poisoning. In our study, during the exposure phase and the non-exposure phase, no positive correlation was found between the average cholinesterase level and the professional age, the clinical signs observed, and the use of PPE. A significant difference was found between the mean cholinesterase level during the exposure, non-exposure and controls. The inhibition of BChE activity during the exposure period and the non-exposure period was higher for some workers indicating that they could be exposed to pesticides during their agricultural activities. In addition, some had higher activity during periods of exposure than non-exposure. It is necessary to sensitize those responsible for this agricultural unit in order to carry out a semi-annual biological evaluation of these workers as recommended by the WHO (1979) for subjects exposed to organophosphates and carbamates. Further studies should be conducted on a larger number of workers in order to have a more representative conclusion.

CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

AYKMA initiated this study and took part in the collection of field data, analysis of the data, discussion and drafting of the manuscript. CC initiated this study and supported the discussion of the results and made corrections to the manuscript. NL participated in data processing. SD initiated this study.

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