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Occupational Exposure of Pesticides on Activities of *Alanine aminotransferase* and γ-Glutamyltranspeptidase in Blood among Vegetables Farmers

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Abstract: Problem statement: Most vegetable farmers are using agricultural chemicals on their farms. Many of these chemicals are used to control pests and are known as pesticides. Pesticides can be considered according to their chemical basis. Most of the more toxic pesticides fall into chemical groups of organophosphates, carbamates and bipyridyls. **Approach:** The general objective to study the level of liver enzymes as a result of being exposed to pesticides among the farmers in Muar, Johor. The specific objectives of the study are to identified relationship between pesticides exposure durations with the liver enzyme level, frequency handling the pesticides and using PPE when application the pesticides, compare the liver enzyme level between exposure group and comparative group and finally identified the factor which can influence the liver enzyme lever at farmer who exposed to pesticides. The cross sectional study on the effect of pesticides to the liver enzymes among farmer at Muar Johor was conducted by using the liver enzymes such as Alanine Aminotransferase (ALAT) and γ-Glutamyltranspeptidase (GGT) as indicators. 92 workers were selected as respondents, where 47 respondents are in the exposed group and the other 45 respondents in the comparative group. Respondents had been selected from farmers in Muar as the exposed group and respondents who from Terengganu Health District Office as the comparative group. The selection of the respondents was made through purposive sampling from list provided. The liver enzymes level was measured by taking blood from respondent. The Automatic Analyzer Hitachi 902 was used in the blood analysis. Two types of the liver enzymes (ALAT and GGT) were analyzed. **Results:** Mean GGT for exposed group was 63.5 Units/L and ALAT was 61.9 Units/L, significantly higher compare to comparative group while the mean of GGT was 20.7 Units/L and ALAT was 23 Units/L. Non-parametric difference Mann-Whitney U test showed there was significant different between exposed and comparative group on GGT ($Z = -6.535$; $p \leq 0.001$) and ALAT level ($Z = -5.315$; $p \leq 0.001$). Five occupational factors have been measured in this study which is years of working, number of pesticide used per day, frequency of handling pesticide per day, pesticide spraying durations and Personal Protective Equipment (PPE) scores. Result showed both GGT and ALAT enzymes level has significant correlations with pesticide spraying durations ($r = 412$; $p = 0.004$ and $r = 0.445$; $p = 0.002$) and Personal Protective Equipment (PPE) scores ($r = -0.397$; $p = 0.006$ and $r = -0.478$; $p = 0.001$). Pesticides spraying durations was the occupational exposure factoring which most influence GGT ($\beta = 0.710$; $p<0.001$) and ALAT ($\beta = 0.574$; $p<0.001$) enzymes level among exposed group after adjustments for all confounders in this study. **Conclusion:** After adjustments of the confounding factors, result shows that frequency of handling pesticide per day and pesticide spraying duration (hours) influence the enzyme GGT level among exposed workers, meanwhile years of working and pesticide spraying duration (hours) influence the enzyme ALAT level among exposed workers.

Key words: Occupational exposure, Alanine Aminotransferase (ALAT), Glutamyltranspeptidase (GGT), Personal Protective Equipment (PPE), vegetables farmers, research ethic, liver enzymes, Statistical Package for Science Social (SPSS), exposed group

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INTRODUCTION

Pesticides are toxic chemicals that are widely used throughout the world. This broad category of compounds includes approximately 600 different active ingredients in use and a far greater number of commercial products. The main application of pesticides is in agriculture, although its use for public health programmes such as malaria or rodent control is also significant in some areas of the world (Garcia, 1998). Agricultural workers are exposed to pesticides primarily through mixing of chemicals, loading into dispensers, application, clean up and disposal of empty chemical containers (Elsebae, 1993; Calvert *et al*., 2003).

Pesticide damage primarily at the nervous system and neurobehavioral functions test methods have been one of the method for detecting this damage in cross sectional workplace research (Bazylewicz-Walczak *et al*., 1999; Rom, 2007; Stephens *et al*., 1995). Other than that, exposures to pesticide can be evaluated by measuring the level of liver enzymes such as Alanine aminotransferase (ALAT) and γ-Glutamyltranspeptidase (GGT) as a biological indicator (Di Lorenzo *et al*., 2003; Altuntas *et al*., 2002; Cheng *et al*., 1999; Giannini *et al*., 1999).

This study focuses on relationship between pesticide exposures with level of liver enzymes among the farmers in Muar, Johor. The specific objectives of the study are to identified relationship between pesticides exposure durations with the liver enzyme level, frequency handling the pesticides and using PPE when application the pesticides, compare the liver enzyme level between exposure group and comparative group and finally identified the factor which can influence the liver enzyme lever at farmer who exposed to pesticides. In agriculture base country like Malaysia, surprisingly there are relatively little of liver enzymes research on workers occupationally exposed to pesticides. In Malaysia, pesticides are typically applied to vegetable crops, palm plantations and tobacco farming. Safety measures are generally poorly applied and workers lack proper knowledge or training in safe handling of these chemicals.

MATERIALS AND METHODS

Research ethic: An official permission letter was obtained from University Research Committee to ensure this study get cooperation from respondent. The purpose of the study was explained to the Vegetables Farmers Society in Muar district, Johor and Health District Office in Kemaman, Terengganu. All respondents in this study were volunteers who signed a statement of formal consent before testing. The consent form was developed according to the international ethical guidelines for biomedical research involving human subjects prepared by the Medical and Health Sciences Research Ethical Committee.

Sampling: This Cross-sectional study using purposive sampling base on inclusion criterion. Forty seven males were recruited from those who are working with pesticide in farm and eligible for inclusion study criteria such as Malay or Indonesian, age in the range of 20 to 40 years old, no medical diagnoses such as diabetes mellitus, liver or kidney disease, immunology disorder, peripheral neuropathy, vitamin deficiency, anaemia and addiction, not alcohol drinker and not smoking and exposed to pesticide more than 5 years. Comparative group are 42 males workers at Kemaman District Health Office and also eligible for inclusion study criteria except they are not occupationally exposed to pesticide.

Instrumentations: Questionnaire was using to obtain the demographic, socio-economic and pesticides exposures among respondents. The interview of questionnaire was conducted through face-to-face interview technique. Certified nurse took a 5 ml sample of venous blood under complete aseptic precautions from 47 exposed and 42 unexposed respondents. Blood samples were sent to certified university laboratory for analysis of Alanine aminotransferase (ALAT) and γ-Glutamyltranspeptidase (GGT) using Hitachi 902 automated analyzer from Boehringer & Mannheim, Germany with standard method reagents (Verplanke *et al*., 2000). Blood samples were collected form all respondents after completion of the questionnaire and health examination.

Data analysis: All data were analyzed using Statistical Package for Science Social (SPSS) version 11.5. Descriptive information generated from all variable. Non-normal distributions of variables for ALAT and GGT were transformed logarithmically, but the distributions are still not normal. Non-parametric Mann-Whitney U tests were used to compare the median of the ALAT and GGT between exposed and unexposed respondents. The Spearman's rho tests were using to define the correlations between occupational exposures with enzymes level. Repeated measurement analysis was performed with the multiple regression model using enter method to define the influence of occupational exposure on level enzymes of exposed workers after adjustments for confounders.

RESULTS

Demographics: There are 92 volunteers respondent involved in this study. Forty-seven of them are exposed to pesticide and 42 are comparative group. The mean age of the comparatives respondents (32.7 years) was slightly older than that of exposed respondents (31.7 years), but the difference was not significant (p=0.714), (Table 1). The mean educational level of the exposed respondents was 9.1 years and the mean of that the comparatives group was 10.5 years. This difference was also not significant (p=0.772). The mean income of the comparatives respondents (RM6449.90) was slightly higher than that of exposed respondents (RM628.70), but the difference was not significant (p=0.457). The mean years of working of the exposed respondents was 11.4 years and the mean of that the comparatives group was 12.7 years. This difference was also not significant (p = 0.684).

Liver enzymes level: Distribution of enzymes GGT and ALAT for exposes group shown in Fig. 1 and 2, meanwhile for comparatives group shown in Fig. 3 and 4. For exposed group, the mean of GGT was 63.5 Units/L and ALAT was 61.9 Units/L, significantly higher compare to comparative group while the mean of GGT was 20.7 Units/L and ALAT was 23 Units/L. All of this data distribution was not normally distributed. Non-parametric difference Mann-Whitney U test showed there was significant different between exposed and comparative group on GGT (p≤0.001) and ALAT level (p≤0.001), (Table 2).

Occupational factors and liver enzymes: Five occupational factors have been measured in this study which is years of working, number of pesticide used per day, frequency of handling pesticide per day, pesticide spraying durations and Personal Protective Equipment (PPE) scores. Result showed both GGT and ALAT enzymes level has significant correlations with pesticide spraying durations (p = 0.004; p = 0.002) and Personal Protective Equipment (PPE) scores (p = 0.006; p = 0.001), (Table 3). Pesticides spraying durations was the occupational exposure factoring which most influence GGT (p<0.001), (Table 4) and ALAT (p<0.001), (Table 5) enzymes level among exposed group after adjustments for all confounders in this study.

Table 1: Socio-economic background for all respondent

	Mean ± SD			
Variable	Exposed (n = 47)	Comparative (n = 45)	t value	p value
Age (years)	31.7±8.06	32.7±10.45	-0.944	0.714
Educational level (years)	9.1±2.99	10.5±2.27	-0.872	0.772
Income (RM)	628.7±86.27	644.9±94.49	-1.083	0.457
Years of working (years)	11.4±6.13	12.7±9.09	-0.788	0.684

N=92

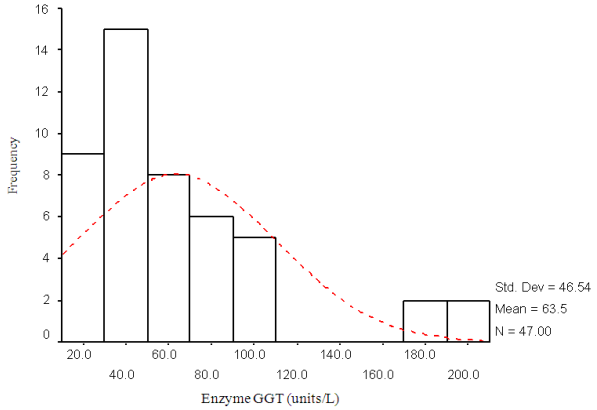

Fig. 1: Distributions of enzyme GGT among exposed respondent

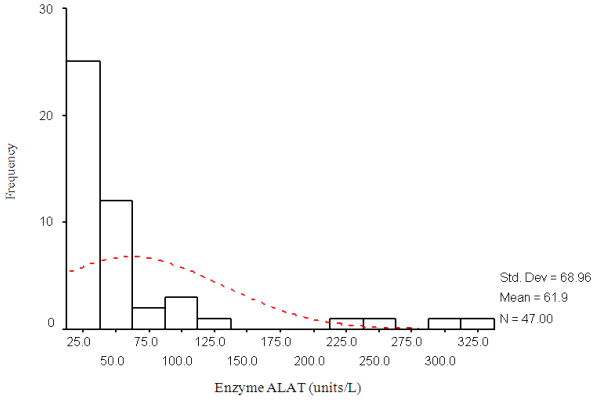

Fig. 2: Distributions of enzyme ALAT among exposed respondent

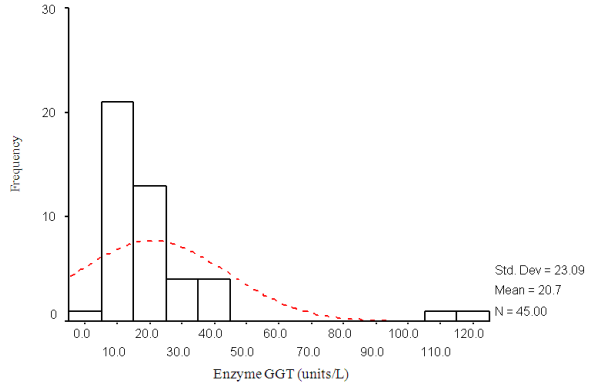

Fig. 3: Distributions of enzyme GGT among comparative respondent

Table 2: Difference test for enzymes level among respondent

Enzymes	[Median] Mean ± SD Exposed (n = 47)	Comparative (n = 45)	Z value	p value
γ-glutamyltranspeptidase (GGT)	[49.0] 63.5 ± 46.54	[15.0] 20.7 ± 23.09	-6.535	≤ **100.0
Alanine aminotransferase (ALAT)	[36.7] 61.9 ± 68.96	[22.1] 22.9 ± 10.87	-5.315	≤ **100.0

N = 92 **: Significant at p ≤ 0.01, comparison using Mann-Whitney U Test

Table 3: Correlations between enzymes GGT and ALAT with occupational factors among exposed workers

Occupational factors	GGT level (Units/L) r value	p value	ALAT level (Units/L) r value	p value
Years of working (years)	0.144	0.333	-0.026	0.863
No. of pesticide used per day	-0.215	0.146	-0.258	0.080
Frequency of handling pesticide per day	0.142	0.341	0.106	0.476
Pesticide spraying duration (hours)	0.412	0.004**	0.445	0.002**
PPE scores	-0.397	0.006**	-0.478	0.001**

N=47 **: Significant at p ≤ 0.01, correlations using Spearman's rho Test

Table 4: The influence of occupational exposure on level enzyme GGT of exposed workers after adjustments for confounders

Independent variable	Level enzyme GGT (Units/L) Regression coefficient (β)	t value	p value
Constant (α)	-147.402	-1.372	0.178
Age (years)	0.353	0.892	0.378
Educational level (years)	-0.198	-1.153	0.256
Household income (RM)	0.411	1.006	0.321
Years of working (years)	-0.850	-1.937	0.060
No. of pesticide used per day	0.085	0.622	0.538
Frequency of handling pesticide per day	0.341	2.427	0.020*
Pesticide spraying durations (hours)	0.710	6.676	< **100.000
PPE scores	-0.159	-1.348	0.186

N=47 *: Significant at p ≤ 0.05; **: Significant at p ≤ 0.01; r = 0.798; R^2 = 0.561 (enter method); F = 8.345; p ≤ 0.001

Table 5: The influence of occupational exposure on level enzyme ALAT of exposed workers after adjustments for confounders

Independent variable	Level enzyme ALAT (Units/L) Regression coefficient (β)	t value	p value
Constant (α)	-190.279	-1.027	0.311
Age (years)	0.653	1.418	0.164
Educational level (years)	-0.156	-0.778	0.441
Household income (RM)	0.274	0.576	0.568
Years of working (years)	-1.121	-2.196	0.034*
No. of pesticide used per day	-0.020	-0.127	0.899
Frequency of handling pesticide per day	0.218	1.336	0.189
Pesticide spraying durations (hours)	0.574	4.635	< **100.0
PPE scores	-0.111	-0.809	0.424

N=47 *: Significant at p ≤ 0.05; **: Significant at p ≤ 0.01; r = 0.713; R^2 = 0.405 (enter method); F = 4.919; p ≤ 0.001

DISCUSSION

Generally this study gets very good corporations from all respondent, exposed and comparative group. Table 1 showed this exposed and comparative group also have been matched successfully with their age (p=0.714), educational level (years) (p=0.772), income (RM) (p=0.457) and years of working (years) (p=0.684).

Results showed that was a significant different between exposed and comparative for GGT and ALAT enzymes level. Mean value for GGT enzyme of exposed group was 63.5 ± 46.54 U/L which is 3 time higher compare to comparative group it was 20.7 ± 23.09 U/L. Same pattern of results for enzyme ALAT, where the mean value for exposed group was 61.9 ± 68.96 U/L meanwhile for comparative group was 22.9 ± 10.87 U/L.

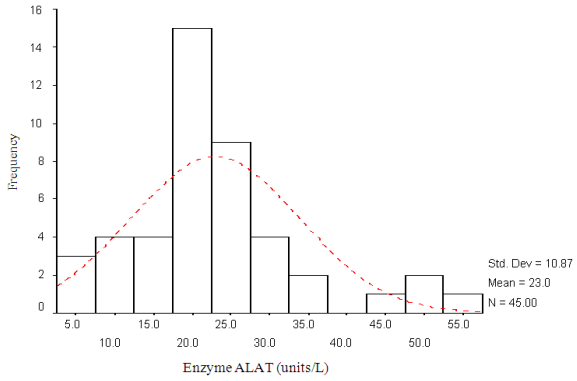

Fig. 4: Distributions of enzyme ALAT among comparative respondent

Increments of liver enzymes are significantly higher among individual who exposed to pesticides and that was the fact supported by many previous studies (Bazylewicz-Walczak *et al*., 1999; Arndt *et al*., 1998; Mage *et al*., 2000). Study Purposely to investigate the effects of insecticides on liver enzymes among sprayer working at Health Department of Kuala Lumpur Municipal Council also founds that AST, ALAT and GGT enzymes are significantly higher in exposed group compared to comparative group. Previous study also found that liver enzymes level among technical and services electronic workers a higher and not normal cause by exposure to organic solvent such as xylene, acetone, benzene isopropanol and others (Upfal, 1992).

This study also found that pesticides spraying durations and total PPE scores factors only were significantly influencing the level of GGT and ALAT enzyme of exposed group. Others factor such as years of working, number of pesticides used per day and frequency of handling pesticides per day was not significantly correlated with level of liver enzymes. Previous study also found that have no significant correlations between durations of organic solvents with increasing of liver enzymes among construction workers, the level of liver enzymes are significantly higher compare to normal populations (Arndt *et al*., 1998). Other study found that years of education, pesticides spraying durations and total PPE scores factors was the major contributors of liver enzymes increment among agriculture workers (Bazylewicz-Walczak *et al*., 1999), there was a significant correlation between years of working with liver enzymes level among environmental disinfestations workers (Tomei *et al*., 1998).

Previous study found that pesticides poisoning was cause through this factor that are frequency of handling pesticides per day, number of pesticides used per day and pesticides spraying durations (Sunding and Zivin, 2000). This study results also found that enzyme GGT and ALAT of exposed group was significantly influence by pesticides spraying durations ($r = 0.412$; $p = 0.004$), ($r = 0.445$; $p = 0.002$) and total PPE scores ($r = -0.397$; $p = 0.006$), ($r = -0.478$; $p = 0.001$) but not others factors.

Others study was design to studies the liver damage cause by toxic waste and pesticides in occupational exposure (Giannini *et al*., 1999; Tomei *et al*., 1998). This study founds that high dose, low dose at long durations and PPE application have a significant correlations with liver deceases.

This study also has the confounding such as demographic and socio-economy factors. In multiple regression statistical analysis after adjustments of the confounding factors, result shows that frequency of handling pesticide per day ($\beta = 0.341$; $p = 0.020$) and pesticide spraying duration (hours) ($\beta = 0.710$; $p<0.001$) influence the enzyme GGT level among exposed workers. Meanwhile this study also found that years of working (years) ($\beta = -1.121$; $p = 0.034$) and pesticide spraying duration (hours) ($\beta = 0.574$; $p<0.001$) influence the enzyme ALAT level among exposed workers, after adjustments of the confounding factors. Other study also found the supportive result which is after adjustment of their study confounding they still get the significant correlation between GGT level with frequency of handling pesticide per day, number of pesticide used per day and pesticide spraying durations (Verplanke *et al*., 2000).

CONCLUSION

In conclusion, this study found there are significant differences of liver enzyme (GGT & ALAT) level between exposed group and comparative group due to pesticides exposure. Other than that this study also covered there are significant correlation between liver enzyme (GGT & ALAT) level with pesticide spraying durations (hours) and PPE score. After adjustments of the confounding factors, result shows that frequency of handling pesticide per day and pesticide spraying duration (hours) influence the enzyme GGT level among exposed workers, meanwhile years of working and pesticide spraying duration (hours) influence the enzyme ALAT level among exposed workers.

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