Assessing the Genotoxic Damage among Farming Community: Identifying and Prioritizing the Associated Risk Factors

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ABSTRACT

Objective: This study aims at determining the association between the genotoxic damage with various risk factors among farmers and the children from the pesticide treated rice farming village in a tropical country.

Method: A face-to-face interview was conducted to examine the potential risk factors of genotoxicity among farmers and the children. The genotoxicity was measured by micronuclei (MN) assay in detecting the chromosome breakage from their buccal mucosal cells.

Result: The findings highlighted that farmers and the children showed significantly (p<0.05) higher MN frequency than their unexposed group. Linear regression analysis of various risk factors among farmers revealed that the chromosome breakage changes in accordance to: individual factors (R²=0.199, p<0.05) and occupational factors (R²=0.122, p<0.05). On the other hand, MN frequency among the children increases significantly (p<0.05) with number of family numbers (who worked as pesticide sprayer) and residential distance from the pesticide-treated farmland. Linear regression analysis of various risk factors among the children revealed that the chromosome breakage changes significantly due to early farming exposure (R²=0.102, p<0.05).

Conclusion: Study emphasizes the need to consider various risk factors which may likely contributing to genotoxic risk. This could help to prioritize the control measures during health risk management among different vulnerable population from long-term and chronic pesticide exposure at the pesticide-treated farming village.

Keywords: Farmer, Children, Pesticide, Risk Factors, Genotoxic Damage

1. Introduction

To date, farmers rely heavily on pesticide to increase their productivity and combat against insect growth to ensure that their production yield is sufficient to supply to the increasing needs by the growing population. In view of this, communities in farming villages are most likely to be exposed to pesticides used. The farmers who work in the farm have their families who live within the pesticide-treated farming village. In other words, not only the farmers, but also the children from the farming village are considered at risk of being exposed to the toxic pesticides mixtures. Past studies highlighted that the potential chronic health effect
of exposures to pesticides mixtures used in the agricultural crop by farmers. The primary concern was related to the genotoxic risk due to their nature of work as a pesticide applicator (How et al., 2015a; Alavanja and Bonner, 2012). Nevertheless, the assessment of genotoxic effect was rarely studied (How et al., 2015c).

Factor analysis conducted by Samanic (et al., 2005) highlighted the necessity to examine the complexity of various risk factors of pesticide-induced health effect over time. When facing the environmental stress, human body cells will first activate a response system that causes the cell cycle to arrest DNA repair mechanism at sufficient time. However, this balance mechanism could be disturbed by individual factors, occupational exposures or the residential setting which in turn caused genomic instability and may induce DNA damage over time (Gerl and Vaux, 2005).

Children’s organs are developing and have immature metabolic pathways compared to adult (Garry, 2004). This makes children uniquely vulnerable to environmental toxicants (Landrigan et al., 2004). In this study, most of the villagers handled family subsistence small-holding farms. Children who live in this farming village might experience an early life of low-levels but cumulative exposure to pesticides mixtures. For instance, they are gradually transformed from mouthing objects and crawling on the floor to children or even adults actively involved in the outdoors and farming activity throughout their childhood life (Carozza et al., 2009).

It is undeniable that farmers are at high risk of exposure to pesticides due to their work nature as a pesticide applicator (How et al., 2013; Alavanja and Bonner, 2012). They are considered as the most susceptible group for genotoxic development. Therefore, the children or family member who lives near pesticide-treated farming area also have the equivalent genotoxic risk. This study is one of the few which examined the potential risk factors (individual, occupation and residential factors) that induce genotoxicity among farmers and children in the farming village.

2. Materials and Method

This is a cross-sectional comparative study sampled during the growing phases of rice farming from September 2012 to January 2013 in the Tanjung Karang district, Malaysia. There were a total of 320 adults studied which consisted of 160 from exposed adult (farmers) and 160 from the unexposed group. A total of 180 school children were recruited, which made up of 95 children from schools in the proximity to the paddy farming areas and 85 of unexposed children from a rural fishing village and about 30-50km from any agricultural areas.

2.1. Risk Characterization

The self-reporting of the risk as perceived by respondents, was based on the questionnaire which consisted of three sections; the individual factor, the work nature (early farming activity), and the residential setting. These risk factors were assessed to associate them with the genotoxic effects.

2.2. Genotoxicity Test (Micronuclei (MN) Assay)

The exfoliated buccal mucosa was collected by using a sterile cytology brush (Gentra Puregene Buccal Cell Kit, Qiagen Ltd., Venlo, Limburg). The micronuclei (MN) assay was used as an internal dosimeter to examine the chromosome breakage (genotoxic damage) among the population who had been exposed to a mixture of pesticides (Kashyap and Reddy, 2012).

The MN originates from acentric chromosomal fragment or whole chromosome which were neither included in the daughter nuclei remaining in the cytoplasm, nor been observed at the interphase as small nuclei. This MN which were found in tissues-specific cells may be used to detect clastogenic and aneugenic compounds (How et al., 2015c). In this study, MN assay was conducted based on the standard protocol described by Thomas and Fenech (2008). The end point was to measure the cells with the presence of MN(s), and was scored based on the cells presented with a main nucleus and smaller nuclei called micronuclei (MN). The MN was usually round or oval in shape and their diameter ranged between 1/3 to 1/10 of the diameter of the main nucleus.

2.3. Statistical Analyses

Independent t-test for independent observations was used to determine the significant difference in MN frequency (per 1000 cells) between the study groups. In addition, simple and multiple linear regressions were used to examine the variability of individual, occupation and residential factors to explain their influence on MN frequency among the study population.

3. Results

Table 1 summarized and compared the MN frequencies in exfoliated buccal mucosal cells between the 2 groups of the adult and children groups. Results showed that the MN frequencies had significant difference (p<0.001) between exposed and the unexposed groups. Farmers showed significantly 3 times higher MN formation.
than the unexposed group. However, for the children, the increase in risk was at least 1.5-2 times significantly higher than the unexposed children.

Table 1: The MN count among unexposed and the unexposed groups

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Children Mean (SD)</th>
<th>Adult Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN count</td>
<td>Exposed 5.06 (2.45) Unexposed 2.92 (1.54)</td>
<td>Exposed 14.48 (4.20) Unexposed 5.47 (1.67)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* p is significant <0.05

3.1. Relationship between risk factors and genotoxic effect.

Table 2 shows the results of a regression analysis to evaluate the potential risk factors contributing to the genotoxic effects. The individual (age, smoking duration and frequency, passive smoker) (p<0.001) and occupational (year of employment) parameters (p<0.001) were shown to significantly influence the MN frequency. This indicated that 19.9% and 12.2% of the variance in MN frequency was explained by the individual and occupational model.

Table 2: Relationship between risk factors and MN count among adults

<table>
<thead>
<tr>
<th>Factors</th>
<th>Predictor(s)</th>
<th>b</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>Constant</td>
<td>8.52</td>
<td>0.015*</td>
<td>0.199*</td>
</tr>
<tr>
<td></td>
<td>Age (year)</td>
<td>0.28</td>
<td>0.006*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>0.01</td>
<td>0.959</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smoke duration</td>
<td>0.19</td>
<td>0.048*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smoke frequency</td>
<td>0.11</td>
<td>0.018*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Passive smoker</td>
<td>-2.47</td>
<td>0.002*</td>
<td></td>
</tr>
<tr>
<td>Occupational</td>
<td>Constant</td>
<td>13.57</td>
<td>0.001*</td>
<td>0.122*</td>
</tr>
<tr>
<td></td>
<td>Year of working</td>
<td>0.12</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Work duration</td>
<td>-0.22</td>
<td>0.631</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Work frequency</td>
<td>-0.09</td>
<td>0.583</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variety of PPE</td>
<td>0.16</td>
<td>0.406</td>
<td></td>
</tr>
<tr>
<td>Residential</td>
<td>Constant</td>
<td>14.44</td>
<td>0.001*</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Distance to farm</td>
<td>0.15</td>
<td>0.759</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration of residency</td>
<td>0.01</td>
<td>0.886</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of pesticide used</td>
<td>0.20</td>
<td>0.319</td>
<td></td>
</tr>
</tbody>
</table>

3.2. Relationship between risk factors and genotoxic effect among the children

Table 3 tabulated the linear regression of these risk factors with MN frequency to show that there was a significant relationship (p<0.05) of the early farm activity exposure to MN frequency among the children. Overall, an approximate of 6.3%, 10.2% and 7.8% of the changes in individual, early farm activity and residential factors influenced the MN frequency among the children.

Table 3: Relationship between risk factors and MN count among the children

<table>
<thead>
<tr>
<th>Factors</th>
<th>Predictor(s)</th>
<th>b</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>Constant</td>
<td>3.13</td>
<td>0.359</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>Age (year)</td>
<td>0.32</td>
<td>0.287</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gender b</td>
<td>-1.17</td>
<td>0.021*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>-0.11</td>
<td>0.222</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parents’ smoker b</td>
<td>0.28</td>
<td>0.596</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other family smoker</td>
<td>1.16</td>
<td>0.034*</td>
<td></td>
</tr>
<tr>
<td>Early Expose</td>
<td>Constant</td>
<td>2.49</td>
<td>0.154</td>
<td>0.102*</td>
</tr>
<tr>
<td></td>
<td>No. family as farmer</td>
<td>0.89</td>
<td>0.009*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Helped in farmland</td>
<td>1.32</td>
<td>0.545</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variety of PPE</td>
<td>-1.07</td>
<td>0.387</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Safety knowledge b</td>
<td>1.21</td>
<td>0.222</td>
<td></td>
</tr>
<tr>
<td>Residential</td>
<td>Constant</td>
<td>14.44</td>
<td>0.036*</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>Residency location b</td>
<td>-0.09</td>
<td>0.736</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distance to school b</td>
<td>0.81</td>
<td>0.036*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transport to school b</td>
<td>0.17</td>
<td>0.781</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Attended pre-school b</td>
<td>-0.10</td>
<td>0.793</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-school distance b</td>
<td>-0.07</td>
<td>0.837</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pesticide storage b</td>
<td>-0.29</td>
<td>0.219</td>
<td></td>
</tr>
</tbody>
</table>

* Simple/Multiple Linear Regression, * Gender (1=Boy, 2=Girl); Passive smoker (1=Yes, 2=No); Helped in Farmland (1=Yes, 2=No); Safety knowledge pursued (1=Yes, 2=No); Residence duration (year); Residence location (1=Paddy farmland, 2=Residence area, 3=Main road, 4=Wood/bushes); Residence distance, meter (1= <1000,2=1000-2000,3= >2000); Transport to school (1=Walking,2=Cycling,3=By motorcycle,4=By car); Attended pre-school (1=yes, 2=no, 3=yes, 4=Not attended), Distance to pre-school, meter (1= <1000,2=1000-2000,3= >2000,4=Not attended); Household pesticide storage (1=Non-user, 2=Backyard, 3=Indoor, 4=Storeroom), * p-value is at significant <0.05.
4. Discussion

One of the most important knowledge gaps was to answer whether individual, occupational (early farm activity) and residential parameters were the contributing factors in the genotoxic variation among farmers and children in the farming village.

4.1. Risk factors and genotoxic effect among farmers

4.1.1. Individual Factors

It was suggested that human body takes years for a healthy cell to evolve and respond to the environmental stimulants until it cumulatively become cancerous cells (Waris and Ahsan, 2006). Thus far, this study suggested that, age has a strong and positive relationship with the genotoxicity developed by farmers, which is consistent with the findings from previous study (How et al., 2015a).

Tobacco smoking is one of the most important and well-documented cause of cancer development (Kashyap and Reddy, 2012; Nersesyan et al., 2011). The nicotine and tar contents in cigarettes may also induce DNA instability in the oral epithelial cells (buccal mucosa cells). Previous studies showed that smoking significantly contributed to the DNA damage as compared to non-smokers (Bhalli et al., 2009). In view of this, Bonassi (et al., 2003) found the association between smoking with MN formation. Second-hand smoke, due to the involuntary smoke inhaled from the smoker among closed family members may act as an additive effect in inducing genotoxic effect. Past studies revealed that second-hand smoke could induce the DNA adducts and causing metabolites of carcinogens in the urine. Besides, the present study showed an overall increase of genotoxic effects among overweight and obese farmers. This is consistent with past studies which revealed a significant decrease in total antioxidant capacity levels, but increase in DNA instability and oxidative stress among the obese population (Bukhari et al., 2010).

4.1.2. Occupational Factors

Occupational factor is known as the main factors which contributed to the variation of genotoxicity among the farm population. In this study, a farmer who worked for more than 10 years had significant increase in MN frequency. Past studies findings showed that the duration of pesticide exposure has effectively affect genotoxicity among farmers due to their work nature (Ergene et al., 2007). Besides, the study showed that, senior or veteran farmers were likely to protect themselves against pesticide contaminated with a variety of personal protection equipment (PPE) as compared to young farmers.

Past studies emphasized the importance of PPE used to prevent pesticide exposure during spraying activity (Costa et al., 2007; Fenske and Day, 2005) and to minimize the incidence of genotoxicity (How et al., 2015a; How et al., 2015c). Nevertheless, this study showed no significant difference between proper and improper PPE worn by farmers. In fact, field observation noted that “improvised-type” of PPE was widely being used by farmers. This included replacing the waterproof protective cover all with worn out long sleeves and pant, or replace respiratory with a handkerchief, etc. The standard personal protection guidelines as recommended by Department of Standards Malaysia are rarely been followed by farmers. The respondents complained that the standard protective cover were uncomfortable in the hot tropical climate and also restricted their movements during spraying.

4.1.3. Residential factors

Information on residential parameters, such as distance to farmland, duration of residence and number of household pesticide used provided some insight on the influence of pesticide exposure in the residential setting. Since most of the farmers have lived in the farming village since they were born, the duration of residence in the farming village were presumed to expose them to pesticide.

However, this study showed no significant relationship between these residential factors with the MN frequency. There was a possibility that the pesticide residue emitted in the residential area were drifted, deposited, sediment, leashed, and drained to distant environment through air, soil and water medium (How et al., 2015b). Nevertheless, an environmental monitoring of pesticide contaminant should be followed to further determine the impact of these residential factors.

In addition, commercial household pesticides used were also examined to identify the additional risk faced by farmers and these children in and around their homes. The commercial pesticide products were found to be transferred into home or stored in the areas which were accessible by other family members (How et al., 2015b). Nevertheless, no association shown in this study might be due to the fact that, most of the farmers interpreted the question “what type of household pesticide used in and around home” differently. In this study, it was observed that, household-used pesticide was kept as the same storage area as the farm-used pesticide.

4.2. Risk factors and genotoxic effect among the children

4.2.1. Individual Factors

Individual factor is of concern as it involves physiological development since the children were exposed to pesticides throughout their childhood. The effects of individual factors, such as age, gender and body weight were
often postulated to correlate with the causes of different diseases from environmental pollutants (Bonassi et al., 2011).

At the age of 9 to 11 years, children experienced specific exposure-related behaviours, especially in the outdoor activities (Garry, 2004). This study observed that age and gender-related exposure pattern were significantly related to children’s exposure, which was consistent with past studies (Bonassi et al., 2011; Wild et al., 2003). This statement was particularly demonstrated by children who showed increased MN frequency with increased age. Boys were also reported to experience significantly higher genotoxicity than girls as also shown by Arcury et al., 2007).

Past studies highlighted that mothers exposed to pesticides prenatally are more likely to have babies with decreased birth weight and length and predisposed latent normal development (Whyatt et al., 2004). In this study, BMI showed no significant correlation with changes in MN frequency. In fact, findings showed that children with normal weight were physically active and aerobically fit for outdoor activity. Therefore, weight became less of a predisposing factor than age and gender in influencing the incidence of MN frequency among the children.

As discussed earlier, it was concluded that cigarette smoke and second-hand smoke were known human carcinogens (Kashyap and Reddy, 2012; Nersesyan et al., 2011). It was hypothesized that children exposed to ETS in their early age might sustain an additive effect of early cancer risk (Neri et al., 2006). This is consistent with the current study which showed that the children who were exposed to ETS from their parent or other family smoker have a higher MN frequency.

4.2.2. Early Exposure to Farm Activity

The traditional culture in agricultural villages where the children were involved in farming activity to reduce the burden faced by their parents is less likely to occur at present. In this study, the children claimed that their family members who worked in the farmland were grandparents, parents or their older adolescence sibling. Finding also showed that the prevalence of the genotoxic risk among the children was significantly associated with the household members who were farmworkers. The genotoxicity found in these children was most likely due to the potential pesticide take-home pathways which might induce indoor household contamination by having additional farm workers (Arcury et al., 2005).

The children who were involved in the farming activity have higher genotoxic risk and this highlighted the effect of early age pesticide exposure. The findings showed a relatively higher genotoxicity effect among children who wore PPE. It is thus speculated that improper PPE is provided since the PPE guidelines were rarely followed by farmers. Results also showed that a child who worked in the farm and had a certain extent of pesticide safety knowledge has a far lesser MN frequency as compared to children who had no knowledge on pesticide safety and handling.

4.2.3. Residential Factors

Past studies showed that the children who live around the agricultural communities were at high risk of exposure to pesticide as they were most likely spend more time in the proximity to pesticide treated farms as their immediate environment which induced off-target contamination (How et al., 2015b, Miswon, et. al,.2015). In this study, children spent at least 6-7 hours per day at school, where the schools were located in proximity to the pesticide-treated farms and were within 2,000 meters in distance from home. In fact, school teachers complained that they could detect the odours of pesticide in the classrooms when the paddy farms were treated with pesticides.

Another finding showed a relatively higher MN frequency found among the children who attended school by car or motorcycle. These children were mostly transported by their parents before and after working at the farm. In these cases, the vehicles were considered as a medium to transport pesticides from farms into homes and children were the mediator being exposed to pesticides from inside the vehicles and from the parents’ work clothes contaminated with pesticides (How et al., 2015b).

A collection of information on household pesticide used from the questionnaire was an attempt to examine the potential additive risk faced by children. A subtle increase of genotoxic effects among the children who reported having pesticide stored in the backyard and the storeroom. This is consistent with previous study which found that children were exposed to pesticides applied to the yards which were then transferred into the house by pets, adults or children (Quandt et al., 2006; Hamzah et al., 2015; Lokhman et al., 2015).

5. Conclusion

In this study, the MN frequency of adult shown to be affected by individual factors, followed by occupational and residential factors. However, children were likely to be affected by early farm exposure, followed by residential factors then the least contributing factor was the individual parameters. Therefore, this study suggested that the predictors of exposure contributed to the genotoxic effects of the study population. It is hoped that these weighted decisions would help in giving alternative explanations and provide
causal interpretation in the genotoxicity risk assessment. A more detailed study was thus proposed to further characterize the complex interaction between adult-children defense mechanisms with genotoxic effect after a prolonged and chronic systemic pesticide exposure.

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CONFLICT OF INTEREST

There is no potential conflicts of interest persists in this conceptual paper, either to the author, contributor or participants.

ETHICAL ISSUES

This study has been approved by the Board of Ethics Committee of the University Research Involving Humans of Universiti Putra Malaysia (JKEUPM: JKPP Nov(12)7)

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