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Review of Genotoxic Effects for Pesticides Exposure among Rural Community in a Farming Village

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Authors' contributions

This work was carried out in collaboration between all authors. Author VH managed the literature searches, data acquisition and drafted the manuscript. Authors ZH, PI and DO designed and conceptualized this paper. Authors SBMT and SMD revised the paper for intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Background: Past studies have suggested that early exposure of farm children to chronic pesticides were likely to establish genotoxic risk that might lead to cancerous cell development later during their adulthood (if not repaired properly).
Objective: This review aims to fill the knowledge gap concerning the genotoxic effect on the rural community in a farming village from exposure to a mixture of pesticides.

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Results: This review paper shows substantial evidence that farmers were vulnerable to pesticide exposure, however, limited evidence shows that children are at an increased or equivalent risk in terms of the genotoxic effect when considering their exposure to the contaminated environment.
Conclusion: This paper summarizes the existing state of knowledge concerning the genotoxic effects from pesticide exposure among farmers and their children, and highlights the genotoxic effect of pesticides as a health risk for future studies.

Keywords: Genotoxic effects; pesticides; children; adult.

1. INTRODUCTION

In Southeast Asia, agricultural industries rely heavily on pesticides to ensure the production yield and profit. Communities that live in the agricultural villages are susceptible to chronic low-level pesticide exposure. Past studies have suggested that early chronic pesticide exposure was likely to establish genotoxic risk in farm children that might later lead to cancerous cell development during their adulthood (if not repaired properly) [1]. However, there are limited reliable databases on the pesticide-related genotoxic effect or cancer risks, particularly in this region. It is a challenge to protect farming adults and children against pesticides due to the underestimation or under-reporting of cases [2].

For agriculture in a rural environment, working and living conditions are interwoven. Adults and children from smallholdings and family subsistence farms are both involved in agricultural activities, where the health effects from pesticide contamination are felt. Recent studies highlighted that if farmworkers who are occupationally exposed to pesticides are grouped as high risk for cancer development, their children living next to the pesticide-treated farmland may also bear the equivalent or increased genotoxic risk [3-5].

1.1 Principle of Micronuclei Assay

Micronuclei (MN) are small, extra nuclear bodies that are formed during mitosis. They contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division. In anaphase, the microtubules are not attached properly to the chromosomes, causing the MN to lag behind and remain encapsulated in a separate nucleus during telophase [6]. Fig. 1 shows the size of MN (1/5 to 1/20 the size of the nucleus), which is morphologically similar to the nuclei after nuclear staining [7].

The MN can be formed through four generally accepted mechanisms: a) the mitotic loss of an eccentric chromosome fragment (clastogenic); b) mechanical consequences of chromosomal breakage and exchange (clastogenic); c) mitotic loss of whole chromosomes (aneugenic); and d) apoptosis.

It has been suggested that the structural aberrations (clastogenic) are the result of either direct or indirect interaction of the test chemical with the DNA, whereas, numerical (aneugenic) aberrations are the result of interference with the mitotic apparatus to prevent normal nuclear division. In other words, the MN is the biomarker that allows the simultaneous evaluation of both the clastogenic and aneugenic effects [5,8].

MN assay has been widely used in pesticide biomonitoring studies to determine the genetic risk [9-13]. This assay is a suitable internal dosimeter for revealing tissue-specific genotoxic damage in individuals who are exposed to the carcinogenic compound [14].

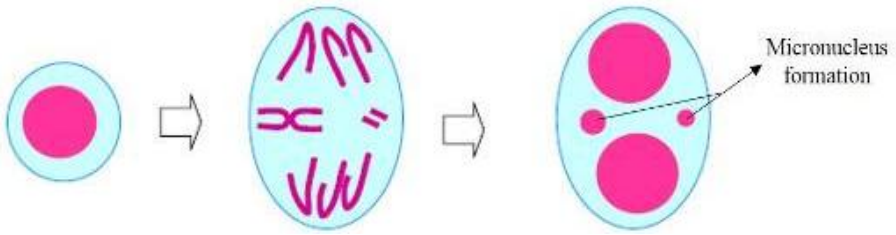

Fig. 1. Micronucleus formation
(Source: Fenech et al. [7])

1.2 Principle of Comet Assay

The Comet assay (also known as Single Cell Gel Electrophoresis Assay, SCGE Assay) is a relatively simple and sensitive technique to detect DNA damage at the individual cell level [14,15].

Briefly, this technique is used to detect the migration of DNA from the individual cell nuclei [16]. Cells embedded in an agarose gel are assembled on a microscope slide and lysed with detergent and high salt to form the nucleoids containing supercoiled loops of DNA linked to the nuclear matrix.

As shown in Fig. 2, the loops containing a break will lose their supercoiling and become free to extend towards the anode under the electrophoresis field, which resembles the shape of a comet [15,17]. The intensity of the comet tail relative to the head reflects the number of DNA breaks. This is followed by viewing the extent of DNA damage under a fluorescence microscope and quantifying with the aid of image analysis software [18].

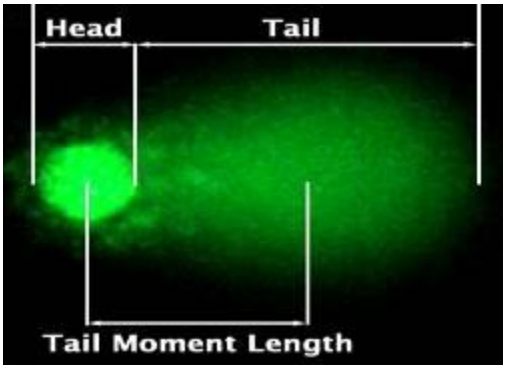

Fig. 2. Comet tail length under fluorescent microscope
(Source: http://www.cellbiolabs.com/comet-assay-kits-and-slide)

Comet assay is a widely used biomarker of oxidative DNA damage, which is used to measure single and double DNA strand breaks [5]. Since DNA damage is an important mechanism that influences cancer development, the evaluation of DNA damage in buccal cells may help provide a good biomarker for early damage in the target tissues [19,20].

2. MATERIALS AND METHODS

Rural children and farmers who originate from the farming village are at increased risk of pesticide contamination from the pesticide-treated farmland due to inevitable environmental and occupational exposure. Therefore, this paper reviewed the available literature to highlight the potential genotoxic effects (comet assay and micronuclei assay) among both rural children and farmers from a farming village.

The PubMed/MEDLINE Resources Guide (http://www.pubmed.gov)/(http://medline.gov), and other electronic databases were used to identify relevant studies in the published literature from 2003-2014. Additional references were selected from reference lists in identified articles. From the identified papers, studies that meet the following criteria were selected:

- A case study, cross-sectional, and case-control study design
- Studies associated with the genotoxic effect (comet tail length and micronuclei) and mixture of pesticides used among farm/rural children and adult farmers/sprayers from farming villages

3. RESULTS AND DISCUSSION

3.1 Micronuclei (MN) Formation from Occupational Exposure

The genotoxic effect to a mixture of pesticides from occupational exposure is influenced by several factors, such as type of work activity, work frequency and duration, and geographic, etc. The effects of these background levels, which may influence the genotoxicity biomarkers, were suggested in previous studies [5,7].

By considering these potential risk factors, Table 1 highlights the inconsistent results of the MN frequency from the mixture of pesticides used among agricultural farmers.

3.2 Micronuclei (MN) Formation from Community Exposure

The MN assay from exfoliated mucosa cells has been widely used to evaluate genotoxicity in children due to its affordability, efficiency, and non-invasive approach [31]. However, limited studies have been conducted to assess the genotoxic effect from pesticide exposure among children [28-30]. The scarce information and knowledge gaps are due to the complex interaction between the environment and the genotype within the matrix of growth-

development, and adaptation causes difficulty in interpretation of the results, particularly when the dynamics of cell division in children is different from that of adults [32].

3.3 Comet Tail Length Formation from Occupational Exposure

Comet assay is a sensitive and rapid method that can be applied to virtually any eukaryotic cell population. The endpoint of this assay can be obtained from a single-cell suspension, as it requires extremely small cell samples (from 1 to 10,000 cells) [16,33]. As shown in Table 2, a growing body of literature has used comet assay to assess the potential DNA damage from pesticide exposure among agricultural workers.

Occupational exposure to environmental toxicants may result in their covalent binding to DNA, which leads to DNA alterations and can be an initial event in the process of chemical carcinogenesis [40]. Nevertheless, past studies that used comet assay as an occupational biomonitoring tool to assess the effects of pesticide exposure had many shortcomings, such as representative sample size, and confounding factors [5,12,17,20].

3.4 Comet Tail Length Formation from Community Exposure

Even though incidences in children from pesticide exposure were widely explicated in past studies, such as leukaemia, neuroblastoma, Wilms tumour, non-Hodgkin lymphoma, etc. [41,42], limited information is available for farm children on their potential genotoxicity effect from the early life exposure to a mixture of pesticides. It has been highlighted that the probability of being subjected to the genotoxicity effect and subsequent development into a cancerous cell may require a period of time after chronic exposure [28]. However, these effects will be doubled if exposure occurs during early life [43].

Table 1. MN Frequency among agricultural farmers from mixture of pesticides (year 2003-2014)

Exposed/Unexposed	Results[a]	Magnitude		Author
51/24	Negative	1.3	-fold decrease	Bolognesi et al., 2004 [21]
64/30	Positive	3.7	-fold increase	Marquez et al., 2005 [22]
54/54	Positive	1.0	-fold increase	Sailaja et al., 2006 [13]
33/33	Positive	3.0	-fold increase	Costa et al., 2007 [10]
69/69	Positive	3.0	-fold increase	Ali et al., 2008 [23]
37/20	Negative	0.3	-fold decrease	Remor et al., 2009 [24]
29/37	Positive	1.0	-fold increase	Bortoli et al., 2009 [25]
70/70	Positive	7.0	-fold increase	Martínez-Valenzuela et al., 2009 [26]
46/48	Positive	2.1	-fold increase	Coskun et al., 2011 [27]
160/160	Positive	2.7	-fold increase	Vivien et al., 2014 [28]

[a]*Positive = statistically significant differences between exposed and unexposed groups*

Table 2. Comet tail length among agricultural farmers from mixture of pesticides (year 2003-2014)

Exposed/Unexposed	Results[a]	Magnitude		Author
19/Nil	Negative	1.3	- fold decrease	Lebailly et al., 2003 [34]
50/56	Negative	1.0	- fold decrease	Piperakis et al., 2003 [19]
45/22	Positive	1.2	- fold increase	Paz-y-Mino et al., 2004 [35]
91/106	Positive	1.1	- fold increase	Liu et al., 2006 [36]
48/33	Positive	1.3	- fold increase	Jors et al., 2007 [37]
134/55	Positive	10.9	- fold increase	McCauley et al., 2008 [38]
37/20	Positive	6.3	- fold increase	Remor et al., 2009 [24]
47/50	Positive	2.3	- fold increase	Bhalli et al., 2009 [39]
33/29	Positive	7.0	- fold increase	Abhay and Gulshan, 2011 [9]
160/160	Positive	1.9	- fold increase	Vivien et al., 2014 [28]

[a]*Positive = statistically significant differences between exposed and unexposed groups*

4. CONCLUSION AND CONTRIBUTION TO KNOWLEDGE

In general, this review is an attempt to summarize the existing state of knowledge about genotoxic effects from pesticide exposure among farmers and their children in an agricultural village. This review paper suggests that this group of farming population were more prone to acquire cancer risk (due to the potential genotoxic effect) throughout their lifetime through living near the agricultural farms. Hopefully these findings will help in giving alternative explanations and provide a causal interpretation of genotoxicity risk assessment.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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