REVIEW ARTICLE

Pesticides Exposure and Biomarkers of DNA damage: A Review

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ABSTRACT

Pesticides are known as potential mutagens as their ingredients may prompt mutations, alter chromosomes, and cause deoxyribonucleic acid (DNA) damage. Biomarker is a common tool used to assess health effects and exposure level. This paper reviewed studies from 2010 to 2020 on pesticides exposure and DNA damage. Seven commonly used biomarkers were comet assay parameters, micronucleus (MN), nuclear buds (NBUD), nucleoplasmic bridges (NPB), 8-hydroxy-2′ -deoxyguanosine (8-OHdG), telomere length, and chromosomal aberrations (CA). The use of these biomarkers was evaluated with their advantages and disadvantages, as well as population exposed. Based on the findings, biomarkers have shown promising results as precursors of DNA damages. The associations between pesticide and DNA damage were equivocal, but most studies showed increment in genetic damages in exposed versus non-exposed population. These biomarkers can serve as predictive marker for risk of initiation and development of cancers or other chronic diseases.

Keywords: Pesticides, Biomarkers, DNA damage, Genetic

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INTRODUCTION

Pesticides are globally used for farm management, pests control, and curbing diseases in agriculture (1). Pesticides have been designed to exert destructive effects on target organisms, and consequently may elicit potential health effects to mankind. Pesticides are capable of inducing mutations, altering chromosomes and causing deoxyribonucleic acid (DNA) damages (2). Pesticides are associated with chromosomal damages in humans and were linked with cancer. Dhananjayan & Ravichandran (3) had reported genotoxicity and cancers were related to occupational exposure of pesticides. Studies have shown the associations of pesticides and cancers including colon cancer (4,5), lung cancer (6,7), and bladder cancer (8). Health effects posed by occupational and environmental exposure of pesticides have been a popular concern due to their potential hazards despite their favourable effects on crops (9). Genetic biomonitoring of populations exposed to pesticides can provide early disease detection and prevention.

Biomarker is often used to determine health effects, assess the exposure, and help in risk prediction (10,11). Pesticides and genotoxicity biomonitoring studies focused mainly on cytogenetic end-points such as chromosomal aberrations (CA), micronuclei (MN) frequency and sister-chromatid exchanges (SCE) even though other biomarkers are currently accessible for genotoxic effects (2). Thus, this paper aimed to review studies related to pesticides exposure and DNA damages among human considering possible biomarkers not limited to cytogenetic end points only. The biomarkers and their parameters reviewed under this study are depicted in Figure 1. Among various biomarkers to detect DNA damages, popularly used markers and justifications for their preference were discussed. The effects of the exposure to pesticides were also reviewed. This review paper summarizes recent findings regarding to pesticides exposure and DNA damages for both occupational and non-occupational exposures.

METHODS

The literatures were searched electronically from Scopus. Keywords “pesticides” and “DNA damage” were searched to find related articles. The title and abstract of each article were read to screen the related studies. The articles were included if they were published in English, human studies, and published between 2010

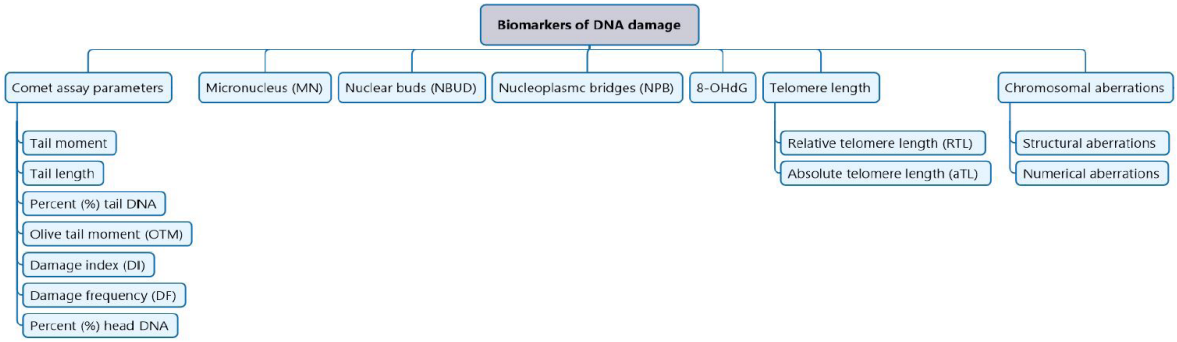

Figure 1: Biomarkers of DNA damage

and 2020. Studies were excluded if they were animals or cell studies.

BIOMARKERS OF DNA DAMAGE

Comet Assay Parameters

Comet assay or single cell gel electrophoresis (SCGE) is a method to measure DNA damage that was established since 1984 (12). Despite 37 years of establishment, comet assay remains as the most popularly used method to measure DNA damage (13), due to its advantages over other methods and its suitability for human studies which are discussed later in this paper. Comet assay is useful in assessing DNA damage for extended and at low level exposure to pesticides (14).

Generally, there are two commonly used comet assay methods, which are neutral comet assay and alkaline comet assay. Neutral comet assay was introduced by Ostling & Johanson (12) and is the fundamental method for other comet assay techniques. Cells with DNA damage demonstrate increase departure of chromosomal DNA from nucleus which create comet shape, hence the name, comet assay (15). However, this neutral method only evaluates double strand break (DSB). This was then modified by Singh, McCoy, Tice, & Schneider (16) and Olive, Banáth, & Durand (17) to alkaline comet assay. In this paper, all reviewed studies used alkaline comet assay method which detect both single and double-stranded breaks.

Comet assay is evaluated by using parameters or image analysis. In reviewed studies, the most frequently used parameters are tail moment, tail length, percent (%) tail DNA, olive tail moment (OTM), damage index (DI), damage frequency (DF), and percent (%) head DNA. Twenty-nine studies showed significantly higher comet assay parameters in exposed groups versus non-exposed groups, one study showed significant increase of comet assay parameter (% tail DNA) in blood taken at the end of cultivation season compared to before cultivation among exposed individuals. Higher % tail DNA was also recorded in those with detectable levels of pesticides compared to subjects with non-detectable levels of pesticides (18). On the other hand, only two studies did not show significant changes in DNA damage; between exposed and non-exposed groups (19); and between pre and post application of pesticides among farmers (20). The probable explanation for this non-significant finding is due to low damage level and ability of the defence mechanism to repair or reverse the damage. A detailed review on comet assay parameters studies is presented in Table I.

Table I: Human epidemiologic studies on pesticides exposure and DNA damage biomarkers

Study population	Biomarkers and parameters analysed	Results	References
Exposed: 134 garlic farmers Non-exposed: 44 non-farmers	Comet assay: tail length, tail moment	(+) There were significantly longer comet tail lengths and tail moments in farmers than non-farmers	(14)
Exposed: 50 urban researchers (exposed to pesticides in laboratory), 50 rural sprayers Non-exposed: 50 urban researchers (control), 50 rural residents	Comet assay: tail length, % tail DNA, tail moment Telomere length: RTL	(+) There were significant increases in tail length, % tail DNA, tail moment among exposed groups than non-exposed groups (+) RTL was significantly shorter among exposed groups compared to non-exposed groups	(15)
Exposed: 19 orchard workers	Comet assay: % tail DNA	(+) % tail DNA was significantly higher in blood taken at the end of cultivation period compared to before cultivation (+) % tail DNA was significantly higher in group with detectable levels of pesticides compared to those with non-detectable levels of pesticides	(18)
Exposed: 186 pesticide sprayers Non-exposed: 22 individuals	Comet assay: tail moment, OTM	(-) There was no significant difference of tail moment and OTM among groups	(19)

CONTINUE

Table I: Human epidemiologic studies on pesticides exposure and DNA damage biomarkers

Study population	Biomarkers and parameters analysed	Results	References
Exposed: 56 farmers (pre and post application)	Comet assay: tail length, tail moment	(-) There was no significant difference in tail length and tail moment for pre and post application	(20)
	8-OHdG level	(-) There was no significant difference in 8-OHdG level for pre and post application	
Exposed: 37 floriculturists Non-exposed: 37 individuals	Comet assay: DF, DI	(+) DF and DI were significantly higher in exposed group than non-exposed group	(24)
Exposed: 63 children Non-exposed: 24 children	MN frequency	(+) There was positive association between MN frequency and pesticides level (-) No significant differences of MN frequency between groups	(25)
Exposed: 41 rural workers exposed to pesticides Non-exposed: 32 individuals	Comet assay: tail moment, tail length, % tail DNA, OTM	(+) % tail DNA, tail moment, and OTM were significantly different between groups	(26)
	MN frequency	(+) MN frequency were 8 times higher in exposed group compared to non-exposed group	
Exposed: 137 farmers Non-exposed: 83 individuals	Comet assay: DI	(+) DI was significantly higher in exposed group compared to non-exposed group	(27)
	MN frequency, NBUD	(+) MN and NBUD frequency were significantly higher in exposed compared to non-exposed group	
Exposed: 13 patients with acute organophosphates (OPs) poisoning (before and after treatment) Non-exposed: 13 healthy subjects	MN frequency, NBUD, NPB	(+) MN frequency was higher in untreated patients than treated patients and healthy subjects (-) No significant difference of NBUD frequency between all groups (+) NPB was significantly higher in untreated than treated patients; and in untreated patients than healthy subjects	(28)
	8-OHdG level	(-) 8-OHdG levels in untreated and treated patients lower than healthy subjects	
Exposed: 90 female tea garden workers Non-exposed: 90 controls (non-tobacco chewers, 70 controls (tobacco chewers)	MN frequency, NBUD	(+) There were significant increases in MN and NBUD frequency in tea garden workers compared to non-exposed groups	(30)
Exposed: 121 agriculture workers Non-exposed: 121 individuals	Comet assay: DI	(+) There was significantly higher DI in agriculture workers compared to non-exposed group	(31)
	MN frequency, NBUD	(+) There was significantly higher MN and NBUD frequency in agriculture workers than non-exposed group	
	Telomere length: RTL	(+) RTL was significantly shorter in farmers than in non-exposed group	
Exposed: 50 farmers Non-exposed: 75 individuals	Comet assay: DI	(+) DI was significantly higher in exposed group than non-exposed group	(32)
	MN frequency, NBUD, NPB	(+) MN, NBUD, and NPB frequencies were significantly higher in exposed group compared to non-exposed group	
Exposed: 129 agriculture workers Non-exposed: 91 individuals	MN frequency, NBUD, NPB	(+) MN, NBUD, and NPB frequencies were significantly higher in exposed group compared to non-exposed group	(33)
Exposed: 68 pesticide sprayers Non-exposed: 43 urban dwellers	MN frequency, NBUD, NPB	(+) MN frequency, NBUD, and NPB were significantly higher in sprayers compared to non-exposed group	(34)
Exposed: 20 children living in farming areas (Aguas Negras, Cabuya, Pelayito, Ceibita) Non-exposed: 13 children living in the city (Monteria	MN frequency, NBUD	(+) MN frequency and NBUD were significantly higher in exposed group living in Pelayito, Aguas Negras, and Cabuya compared to non-exposed group	(35)

CONTINUE

Table I: Human epidemiologic studies on pesticides exposure and DNA damage biomarkers (CONT.)

Study population	Biomarkers and parameters analysed	Results	References
Exposed: 188 agriculture workers (94 workers exposed to mixture with OPs, 94 workers exposed to mixture without OPs) Non-exposed: 50 non-agriculture workers not exposed to pesticides	MN frequency, NBUD	(+) There were significant differences of MN frequency in group exposed to OPs compared to non-exposed group (+) NBUD was significantly higher in group exposed to OPs compared to group that is not exposed to OPs and the non-exposed group	(36)
Exposed: 81 soybean farm workers Non-exposed: 46 office employees	Comet assay: DI, DF	(+) There were significant increases of DI and DF in exposed compared to non-exposed group	(37)
	MN frequency, NBUD	(+) MN frequency and NBUD were significantly higher in exposed compared to non-exposed group	
Exposed: 30 agriculturists Non-exposed: 30 individuals	NPB	(+) NPB frequency were significantly higher in exposed group compared to non-exposed group	(40)
	Telomere length: RTL	(-) There was no significant difference of RTL between groups	
Exposed: 50 mother-infant pairs	Comet assay: OTM	(+) OTM was significantly higher in umbilical cords than in mothers	(41)
	MN frequency, NPB	(+) MN frequencies were significantly higher in mothers than in umbilical cords (-) No significant differences of NPB in mothers and in umbilical cords	
Exposed: 568 cancer-free participants	Telomere length: RTL	Cumulative used pesticides: (+) 2,4-D, diazinon and butylate were significantly associated with shorter RTL (+) Alachlor was significantly associated with longer RTL Recent used pesticides: (+) Malathion was significantly associated with shorter RTL (+) Alachlor was significantly associated with longer RTL	(42)
Exposed: 180 long-term workers exposed to omethoate Non-exposed: 115 healthy controls	Telomere length: RTL	(+) There were significant differences RTL between exposed group and non-exposed group	(43)
Exposed: 1234 pesticide applicators (commercial and private applicators)	Telomere length: RTL	(+) There were significantly shorter RTL in commercial applicators than private applicators (+) There were significantly shorter RTL in Iowa applicators than North Carolina applicators	(44)
Exposed: 62 tobacco farmers Non-exposed: 62 individuals	Telomere length: aTL	(+) aTL was significantly shorter in exposed group than non-exposed group	(45)
Exposed: 268 children	8-OHdG level	(+) 8-OHdG levels were higher in children with high urinary metabolite level compared to those with low urinary metabolite level	(55)
Exposed: 80 sprayers Non-exposed: 85 rural residents, 121 city residents	8-OHdG level	(+) Pesticide sprayers had significantly higher levels of 8-OHdG compared to non-exposed group	(56)
Exposed: 67 farmers Non-exposed: 67 individuals living nearby	8-OHdG level	(+) There were significant increases in 8-OHdG level in exposed group compared to non-exposed group	(58)

CONTINUE

Table I: Human epidemiologic studies on pesticides exposure and DNA damage biomarkers (CONT.)

Study population	Biomarkers and parameters analysed	Results	References
Exposed: 31 pesticides applicators Non-exposed: 34 individuals	8-OHdG level	(-) There was no association between pesticides and 8-OHdG level	(59)
Exposed: 40 horticulture farmers Non-exposed: 40 individuals	8-OHdG levels	(-) There was no significant difference in 8-OHdG level between farmers and non-exposed group	(60)
Exposed: 76 agriculture farmers Non-exposed: 53 individuals	CA: metaphases, monochromatid breaks, isochromatid breaks, dicentric chromosomes	(+) Metaphases, monochromatid breaks and dicentric chromosomes were significantly higher in exposed group compared to non-exposed group	(65)
Exposed: 97 farmers Non-exposed: 55 individuals	MN frequency	(+) There were significant increases in MN frequency in exposed group than in non-exposed group	(66)
	CA: discentric ring, tricentric ring, acentric ring, terminal deletion, interstitial deletion	(+) There were significant increases in CA frequency in exposed group than in non-exposed group	
Exposed: 85 farmers using pesticides Non-exposed: 36 organic farmers, 61 healthy individuals living in the same area	Comet assay: % tail DNA	(+) There were significant increases of % tail DNA in pesticide farmers compared to organic farmers and healthy individuals	(67)
	MN frequency	(+) There were significant increases of MN frequency in pesticide farmers compared to organic farmers and healthy individuals	
	Total CA (number of aberrations per 100 cells, excluding gaps)	(+) There were significant increases of total CA in pesticide farmers compared to organic farmers and healthy individuals	
Exposed: 30 pesticide applicators Non-exposed: 22 individuals	Comet assay: tail moment	(+) There were significant increases in tail moment of exposed group than non-exposed group	(68)
	MN frequency	(+) There were significant increases in MN frequency of exposed group than non-exposed group	
Exposed: 32 rural workers	Comet assay: DI, DF	(+) DI and DF were significantly higher in exposed than in non-exposed group	(69)
Non-exposed: 31 individuals	CA: chromatid breaks, chromosome breaks, polyploidy cells, endoreduplication	(-) No significant difference of CA frequency between exposed and non-exposed group	
Exposed: 70 exposed workers Non-exposed: 70 individuals	Comet assay: % tail DNA, tail moment	(+) There were significantly higher % tail DNA and tail moment in exposed group than non-exposed group	(70)
Exposed: 43 children born and live in agriculture area	Comet assay: tail length, tail moment, OTM, % tail DNA, % head DNA	(+) There were significant differences of tail length, tail moment, OTM, % DNA tail and % DNA head between groups	(71)
Non-exposed: 41 control children	MN frequency	(+) There was significantly higher MN frequency in exposed group than non-exposed group	
	CA: chromatids and chromosome gaps, acentric fragments, chromosome and chromatids breaks, endoreduplications	(+) There were significant differences of chromatid breaks and endoreduplications between groups	
Exposed: 77 tea garden women workers Non-exposed: 66 control women	Comet assay: tail length, % tail DNA, tail moment, OTM	(+) There were significant increases in tail length, % tail DNA, tail moment, and OTM of exposed group than non-exposed group	(72)
Exposed: 69 women picking cotton Non-exposed: 69 women	Comet assay: tail length	(+) Tail length was significantly higher in exposed group than non-exposed group	(73)
Exposed: 22 agriculture workers Non-exposed: 24 individuals living in the same area	Comet assay: Head length, tail length, tail moment, % tail DNA, % head DNA, DI	(+) Tail length, % tail DNA and tail moment were significantly higher in exposed group than non-exposed group	(74)
Exposed: 107 males who had drunk well and/or tap-water (conventional agriculture workers, organic agriculture workers, non-agriculture workers) Non-exposed: 40 males who had consumed only bottled water (non-agriculture workers)	Comet assay: visual score, DNA strand breakage	(+) DNA damage was significantly higher in tap water consumers than water bottled consumers (+) DNA damage was significantly higher in agriculture workers than non-agriculture workers	(75)

CONTINUE

Table I: Human epidemiologic studies on pesticides exposure and DNA damage biomarkers (CONT.)

Study population	Biomarkers and parameters analysed	Results	References
Exposed: 51 agriculture workers Non-exposed: 50 individuals	Comet assay: tail length, % tail DNA, tail moment	(+) Tail length, % tail DNA and tail moment were significantly higher in exposed group than non-exposed group	(76)
Exposed: 30 pilots used to apply pesticides Non-exposed: 30 individuals	MN frequency	(+) MN frequency was significantly higher in exposed group than in non-exposed group	(77)
Exposed: 161 community health agents Non-exposed: 88 individuals	Comet assay: OTM	(+) There was significant increase in OTM of exposed group compared to non-exposed group	(78)
Exposed: 77 tobacco harvesters Non-exposed: 60 individuals	Comet assay: DF, DI	(+) DF and DI were significantly higher in exposed group compared to non- exposed group	(79)
	MN frequency	(+) MN frequency was significantly higher in exposed group compared to non-exposed group	
Exposed: 111 agricultural workers Non-exposed: 60 individuals	Comet assay: tail length	(+) Tail length was significantly higher in exposed group compared to non-exposed group	(80)
	MN frequency	(+)MN frequency was significantly higher in exposed group compared to non-exposed group	
Exposed: 180 *orang asli* children living in agriculture area	Comet assay: tail length	(+) Children with detectable metabolites have longer tail length than children with undetectable metabolites	(81)
Exposed: 37 female farmers, 34 farmer's children Non-exposed: 35 female adults, 38 unexposed children	MN frequency	(+) There were significant differences in MN frequency between exposed farmers and unexposed female adults, and farmers' children and unexposed children	(82)
Exposed: 20 farm owners P0: period without pesticide application, few weeks after last use P4: intensive spraying period Non-exposed: 29 non-farmers	Comet assay: OTM	(+) There was significant difference in OTM between P0 and P4 (+) DNA damage was significantly higher in farmers (P4) than in non-farmers	(83)
Exposed: 100 agriculture workers Non-exposed: 100 individuals	MN frequency	(+) There was significant increase of MN frequency in exposed group compared to non-exposed group	(84)
Exposed: 30 tobacco farmers (3 times sampling: off-season, pesticides application, leaf harvest) Non-exposed: 30 individuals	Comet assay: DI, DF	(+) There were significant increases in DI and DF of tobacco farmers (at all crop times) compared to non-exposed group	(85)
	MN frequency	(+) There were significant increases in MN frequency of tobacco farmers (off-season) compared to non-exposed group	
Exposed: 210 farm workers Non-exposed: 50 individuals	Comet assay: tail length	(+) There were significant differences in tail length between groups	(86)
Exposed: 33 pesticides users Non-exposed: 29 non-users	Comet assay: % tail DNA, tail moment, OTM	(+) % tail DNA, tail moment and OTM were significantly higher in exposed than in non-exposed group	(87)
Exposed: 50 agricultural pilots Non-exposed: 17 individuals	MN frequency	(+) MN frequency was significantly higher in exposed group compared to non-exposed group	(88)
Exposed: 23 exposed workers Non-exposed: 22 individuals	MN frequency	(+) MN frequency was significantly higher in exposed group compared to non-exposed group	(89)

CONTINUE

Table I: Human epidemiologic studies on pesticides exposure and DNA damage biomarkers (CONT.)

Study population	Biomarkers and parameters analysed	Results	References
Exposed: 115 community health workers spraying pesticides Non-exposed: 115 individuals	Comet assay: tail moment	(+) There was significantly higher tail moment in exposed group compared to non-exposed group	(90)
Exposed: 40 pesticides workers Non-exposed: 27 individuals	Comet assay: DI, DF, % tail DNA	(+) DI, DF, and % DNA in tail were significantly higher in exposed than in non-exposed group	(91)

Micronuclei (MN)

Micronuclei (MN) are structures with chromatin in cytoplasm surrounded by a membrane with no link to the nucleus (21). Micronuclei occurs when a chromosome becomes fragmented or lagged and were not included in their main daughter nuclei after mitosis (22). Micronuclei is an extensively used biomarkers in genotoxic and cytogenetic damage studies. In this paper, MN is the second popular biomarkers to assess genotoxicity arising from pesticides exposure. The most commonly used and reliable protocol is cytokinesis-block (CBMN) assay (23). CBMN assay is sensitive as it solely measures MN that has completed nuclear division (22). This assay can measure another DNA damage parameters such as nuclear bud (NBUD) and nucleoplasmic bridge (NPB) (23).

Twenty four out of 26 studies showed significant increases in MN frequencies in exposed group compared to non-exposed groups, while only two studies did not find any significant differences in MN frequencies between groups (24,25). Although MN frequency was not significantly higher in exposed group, it was noted that MN frequency showed a positive association with pesticide exposure (25). Workers exposed to pesticides were reported to have eight times higher MN frequency than control group (26). These findings indicates that pesticides play a vital role in DNA damage by producing MN. The presence of MN reflects genotoxic exposure and chromosomal damage (27). The replication of damaged cell will continue if the damage is not repaired or if the damaged cell is not removed; which will lead to alteration of cell functions (25). It was reported that MN and NPB frequencies were recovered after being treated, notwithstanding MN frequency in exposed group was still higher than control subjects (28). A detailed review on MN studies can be referred in Table I.

Nuclear Bud (NBUD)

The term 'nuclear buds' was first used in 1998 for exfoliated human cells and is said to be a precursor of MN (29). Nuclear bud is the removal of nuclear material by budding from the main nucleus (30). Nuclear bud has a similar morphological structure with MN except its close proximity to the nucleus and its attachment to each other by a nucleoplasmic connection (22). Nuclear buds can be measured using the same method as MN and NPB which is CBMN assay (23). This method is unique, versatile, comprehensive and selective because it measures multiple pathways of genomic instability.

Nuclear buds were found to be significantly higher in exposed group compared to non-exposed group according to 9 out 11 studies (27,30–37). NBUD formation was suggested to form after fusion bridge cycles break and it indicates gene amplification (34,38). It was also proposed that the increase of NBUD frequency cause the production of chromatin loops in peripheral nucleus (27). Notwithstanding, Gundogan et al. (28) and Wilhelm et al. (24) did not find significant changes of NBUD in their studied populations. Wilhelm et al. (24) justified that 70 % of floriculturists used at least one type of PPE and 56.8 % of them used a mask. This reflects the effectiveness of PPE in prohibiting DNA damage effects from pesticides exposure. A detailed review on NBUD studies is presented in Table I.

Nucleoplasmic bridge (NPB)

Nucleoplasmic bridge (NPB) is another biomarker which is closely related to MN and NBUD. It is sensitive and has a lower background level than MN (39). Nucleoplasmic bridge is formed when centromeres of dicentric chromosomes are attracted to cell's opposite poles during anaphase (22,38). Another suggested mechanism is telomere-end fusion which is caused by premature telomere shortening and is often associated with occupational exposure (40). The purpose of scoring NPB is to understand chromosomal rearrangements, poor repair and/or telomere fusion (36). It measures genetic damage from misrepaired DSB, telomere-end fusion, failed sister chromatid separation, or others that cannot be scored through MN (22).

Among NPB studies, five out of six studies reported that NPB frequency was significantly higher in exposed group compared to non-exposed group (28,31,32,34,40). On the other hand, Alvarado-Hernandez et al. (41) did not find significant difference of NPB among mother-infant pairs who were living in agricultural area. These mother-infant pairs were not involved in pesticides handling, thus explaining why they did not have significant NPB

level compared to those who were occupationally exposed to pesticides. A detailed review on NPB studies is summarized in Table I.

Telomere length

Telomere is a particular structure at the end of chromosomes which consists of repeated DNA sequences (TTAGGG)n (40). Telomere length can be measured in two manners; relative or absolute. Six studies reviewed in this paper measured relative telomere length (RTL) (15,31,40,42–44) and only one study measured absolute telomere length (aTL) (45). Relative telomere length is introduced by Cawthon (46) and is considered as one of the gold standard methodologies in measuring RTL. Cawthon (46) used oligonucleotide primers while O'Callaghan, Dhillon, Thomas, & Fenech (47) introduced a method to measure aTL by using oligomer standards.

Significant differences of telomere length were found between exposed and non-exposed groups in majority of the studies (15,31,42–45). Andreotti et al. (42) reported a significant association of different types of pesticides with both longer and shorter telomere lengths. Out of 7 studies reviewed for telomere length, only one study found no significant difference of telomere length between the exposed and non-exposed group (40). A detailed review on telomere length studies is summarized in Table I.

The impact of pesticides on telomere length were inconsistent. Changes in telomere length due to pesticides exposure can either be lengthened or shortened depending on the types of pesticides exposed. Shortened telomeres were observed in cancer cases while longer telomeres were detected as an initial response of low-dose treatment (44). Changes in telomere length suggest that exposure to carcinogenic pesticides may promote tumour growth or early stage of carcinogenesis in humans (48). Many studies have reported that both shorter and longer telomeres have associations with cancer (49–51).

Some pesticides may change cell cycle, therefore it is likely that pesticides may disrupt the correct cell cycle hence contributing to elevation of DNA damage and telomere shortening (31). In addition, telomeres are very sensitive to damage caused by oxidative stress as they consist of repetitive DNA with high guanine content (44,52). Guanine is highly prone to oxidation and therefore generating 8-oxoguanine (8-oxoG), which furthermore susceptible to oxidation (53). Thus, explaining how pesticides can induce changes in telomere length.

8-hydroxy-2′-deoxyguanosine (8-OHdG)

8-hydroxy-2′-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage due to reactive oxygen species (ROS) (54). It is one of the most frequently observed base modifications known to occur (55). It has been suggested as a biomarker that link the oxidative stress and chronic diseases (20,55,56). Oxidative attacks consist of exogenous and endogenous; where exogenous correspond to environmental sources of oxidation while endogenous correspond to natural mechanisms such as metabolic processes (57). Endogenously induced DNA lesions can reach a higher level of cells and tissues mutations than exogenous lesions. (57).

The results of 8-OHdG levels in population exposed to pesticides are inconsistent. Three studies reported significant increase of 8-OHdG in population exposed to pesticides compared to non-exposed group (55,56,58), while four studies did not find changes in 8-OHdG levels or their associations with pesticides exposure (20,28,59,60). The elevated level of 8-OHdG in exposed groups indicates the presence of oxidative stress. Oxidative stress occurs as a result of imbalance level of free radicals and oxidants compared to antioxidants (20). It had been proposed as a promotion mechanism of chronic diseases and carcinogenesis due to pesticides exposure (56). In contrary, the non-association between 8-OHdG levels and pesticides exposure were because antioxidant defence managed to fight free radicals and their toxic effects were not great enough to induce DNA damage (60). In addition, it might be due to uncontrolled potential confounders such as physical activity and diet which may interfere with the results (59). A detailed review on 8-OHdG studies is presented in Table I.

Chromosomal aberrations (CA)

Chromosome aberrations is defined as chromosome abnormality in its distribution, number, structure, or arrangement (61). The distortion of normal cellular processes such as DNA replication, DNA repair, transcription, and cell division cause these aberrations to occur (62). There are two types of CA which are structural aberrations and numerical aberrations. Structural aberrations are caused by improper repair of DNA breaks leading to changes at the nucleotide or chromosomal level, while numerical aberrations are results of chromosomes segregation errors in mitosis (63). It is assumed that structural and numerical aberrations occur independently (64). Thus, each of them can be measured individually or as a combination.

Four studies found significant differences in CA where exposed groups had higher abnormalities than non-exposed groups (65–68). Contrarily, Paiva et al. did not find significant differences in chromatid breaks, chromosome breaks, polyploidy cells and endoreduplication between both groups (69). Besides, no significant difference in CA frequencies were found in subjects with prolonged pesticides exposure. They explained the DNA impairment due to pesticides in their research was not extensive enough to cause mutations or damaging chromosomes (69). A detailed review on CA studies is summarized in Table I.

ADVANTAGES AND DISADVANTAGES BETWEEN DIFFERENT BIOMARKERS OF DNA DAMAGE

Biomarkers are chosen depending on several factors such as types of cells, types of damage and the level of damage to be assessed. Comet assay parameters is popularly adopted biomarker because of its sensitivity, requirement of a small sample size and better selectivity at a single cell level (14). It is often used to detect DNA breaks. Micronuclei test is another reliable and sensitive method but unlike comet assay, it requires nuclear division. It is a biomarker used to assess aneugenic and clastogenic effects (66).

Chromosomal aberration can identify chromosomal damages from structural to numerical abnormalities, however, the process is rather complex. Nucleoplasmic bridge would be a good biomarker of choice to measure of chromosome breakage and rearrangement that cannot be measured by MN assay (22), while NBUD is a suitable biomarker for gene amplification (38). 8-OHdG is a good biomarker to detect oxidative damage due to oxidative stress (14). Likewise, telomere length is also closely related to oxidative stress, thus it is a choice of predictive biomarkers for detecting oxidative stress and senescence. The advantages and disadvantages of each biomarker are listed in Table II**.**

Table II: Summary of advantages and disadvantages of biomarkers

Biomarkers	Advantages	Disadvantages
Comet assay	• Simple • Low cost • Short time of analysis • Small number of cells per sample (<600) • Flexible and adaptive • Identification of damage at single cell level • Direct visualization of DNA damage • No radioactive labelling required • No cell cultivation required • Measure damage of long term exposure at low level	• DNA breaks during apoptosis is too small to be detected • Require control cells due to possible variation during electrophoresis
MN assay	• Simple • Low cost • Short time of analysis • High reliability • Objective results • Can be used on any eukaryotic cells • Automatic scoring • Can be assessed together with NBUD and NPB	• Require cell division • Not applicable for all structural chromosome aberrations (only detect acentric fragments)
NBUD	• Reflect gene amplification • Short time of analysis • Dose dependent • Can be assessed together with MN and NPB	• Require cell division

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Table II: Summary of advantages and disadvantages of biomarkers (CONT.)

Biomarkers	Advantages	Disadvantages
NPB	• Short time of analysis • Low background frequency • Not affected by genders • Direct measurement of asymmetrical chromosome rearrangement • Dose dependent • Can be assessed together with MN and NBUD	• Require cell division
Telomere length	• Different types of methods to choose • Low cost • Available in all linear eukaryotic chromosomes • Requires small number DNA (as low as 100 pg for quantitative fluorescence *in situ* hybridization (QFISH) and single telomere length analysis (STELA) • High accuracy	• Telomere length and rate of change are heterogeneous among species • Require high quality of DNA • Some techniques (quantitative polymerase reaction and telomere restriction fragments) requires a lot of materials and kits • Labour intensive • Require extensive technical understanding
8-OHdG	• Reflect nucleobase mutations • Reflect oxidative damage at specific site and sampling time • 8-OHdG in urine and blood can indicate average rate of oxidative damage for whole body • A stable product and does not undergo metabolism • Does not require enzymatic digestion	• Require high selectivity antibody for 8-OHdG to avoid interference of other compounds • Sensitive sample preparation
CA	• Able to identify all types of chromosome mutations • High sensitivity	• Complex procedure • Time consuming • Require cell cultivation for metaphase • Require high skill personnel • High cost • Subjective results • No automatic scoring

EXPOSED POPULATION

Humans may be exposed to pesticides during occupational and non-occupational activities. Agriculture workers (farmers, pesticide sprayers, harvesters, floriculturists, horticulturist, pesticide workers, and greenhouse workers) are the most studied population as they are exposed to pesticides directly from preparation and application. However, there are

also non-agriculture workers such as community health workers, rural workers, and airplane pilots who are exposed to pesticides (26,69,77,78,88,90). The DNA damage perceived among exposed group may be arising from their exposure to pesticide mixtures. Occupational exposure to pesticides may incite DNA damages, which could be a preliminary predictors in carcinogenesis (15,92).

While general population may not be exposed to pesticides due to occupations, the risk of exposure due to environmental factors still exists especially if they are living near the agriculture area. Another population of concern is the children because they have particular vulnerability to environmental toxics and their levels of detoxification, DNA repair process, as well as cell proliferation may also differ from adult (71). Despite not being involved directly in agriculture activities, living in the agriculture area or having parents or family members who are agriculture workers and exposed to pesticides may increase the risk. Few studies have shown significant DNA damage among children exposed to pesticides (35,55,71,81,82). Since genetic materials can be inherited from parents to children, it is important to assess the genetic damage in children of farmers or those who are living in the farming area.

TYPES OF SAMPLES

In epidemiological studies involving human, it is extremely important to decide on what samples should be collected from respondents. Due to ethical concern, less invasive methods are highly encouraged and preferred to protect respondent's safety. However, it is not always the case. Other factors such as cost, quantity of samples, and information that can be obtained from the samples should be taken into consideration.

While blood sample collection is considered as invasive, it is still widely used in human studies. The advantages of blood analysis include providing evidence of exposure and give an indication of the body burden of pesticide residues (93). Nevertheless, since the method is invasive, it may reduce response rate because respondents might refuse to provide blood sample. Blood sampling requires trained and certified medical personnel which leads to higher cost of sampling.

Buccal sample is an alternative method to obtain DNA. The prime advantage of buccal sampling is that it is a non-invasive procedure, thus it has very minimal risks to respondents. Consecutively, researchers may attract more participants and increase the response rate. Buccal cells can be collected easily either by the respondents or by researchers without help from authorized medical staffs. Therefore, sampling cost can be reduced. Buccal cells are frequently used in MN assay compared to comet assay because buccal cells are suitable in lower pH; where they are resistant to lysis which is a crucial step in comet assay (94).

Urine is another non-invasive biological sample that can be used to measure these biomarkers; however, in this review, it was only used to measure 8-OHdG. Urine is used in detection of 8-OHdG because it is one of best specimens that reflect total modification of body DNA (95). Besides, urine is also a stable sample and can be stored at room temperature for immediate analysis or up to 2 years when stored at −80 °C (95).

Although there are variety of samples available to be used in detection of biomarkers, blood sample remains the most preferred samples. Despite being invasive, blood is a universal sample that can measure not only said biomarkers but also can be used for many other tests. Thus, it is easier to collect blood for one time and use it for multiple analysis instead of collecting different sample types for different analysis.

CONCLUSION

The results of genetic damage due to pesticides exposure are inconclusive as there were contradictory results. It is difficult to impute the genotoxicity to a single compound due to exposure to mixtures of pesticides. Nevertheless, most of the studies reviewed in this paper agreed that significant DNA damages were found among those who are exposed to pesticides. Different types of biomarkers have shown promising results as predictors of DNA damages. These biomarkers can serve as a predictive marker for the risk of initiation and development of cancers or other chronic diseases. Assessing genotoxic with these biomarkers is useful and essential to ensure for early detection and diseases prevention. However, it is not plausible to relate health outcomes with single biomarkers as they have their own unique functions. Each biomarker is capable to detect different types of damage and abnormalities, making great variations biomarkers are available for DNA damages.

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