REVIEW ARTICLE

Minimally Invasive Sampling Sources for Quality and Quantity of DNA for Telomere Length Measurement: A Review

Alia Azwa Abd Rahman[1], Yu Bin Ho[1], NorShariza Nordin[2], Eugenie Sin Sing Tan[3],Rozaini Abdullah[1,4]

[1] Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2] School of Healthy Aging, Medical Aesthetics and Regenerative Medicine, Faculty of Medicine and Health Sciences, UCSI University, 56000 Cheras, Wilayah Persekutuan Kuala Lumpur
[3] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4] Natural Medicines and Products Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

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

Corresponding Author:
Yu Bin Ho, PhD
Email: yubin@upm.edu.my
Tel: +603-86092955

INTRODUCTION

Telomere is a DNA-protein structure consists of repeated tandem sequences, TTAGGG, located at the end of eukaryotic chromosome (1). Telomeres serve as protective cap at the end of chromosome from nucleotide loss during replication (2). It is important for cellular replication where the loss of telomere will result in the inability of cells to replicate hence senescence. Telomerase plays a role as telomere length maintenance which is crucial for cell immortalisation and tumorigenesis (3). Telomere shortening happens each time of DNA replication (4) that could be caused by various mechanisms. These include end-replication problem, end processing and oxidative DNA damage (5). The contributions of these different mechanisms are still unclear, but oxidative stress has been identified as the major cause of telomere shortening (6).

Since Cawthon published the first qualitative Polymerase Chain Reaction (qPCR) for telomere study (7), around 3168 studies cited this paper until January 14, 2022 (data obtained from Google Scholar). Previous studies (8,9) found the different sources of samples influencing the telomere length which varied in DNA quality and quantity due to variation of tissue properties in samples. Although shorter telomere was found in leukocytes in venous blood (VB) compared with muscle (8) and testis (9), a strong correlation was found between TL in blood with another 15 tissue (nerve, brain, artery, skin, lung, colon, etc.) which support the use of whole blood as a proxy of most tissue for TL measurement in epidemiological studies (9). When interpreting TL data from blood and saliva, it is vital to consider a variable number of different cell types and different cell ratios (10). For instance, blood mainly contains leukocytes

as their source of DNA while saliva contains both leukocytes and epithelial cells (11). It is crucial to decide what samples should be chosen from respondents for epidemiological investigations involving humans. Due to ethical concerns, samples obtained from less invasive procedures are strongly encouraged and favoured to protect responder safety. To the best of our knowledge, most of the telomere length measurement studies use venous blood where its withdrawal may cause pain on site of injection, although a large volume of blood can be drawn. It is suggested that pre-analytical variables such as DNA extraction method, sample storage and assay location influence the reproducibility of qPCR relative telomere length measurement (12). In addition, storage and transportation of samples before extraction could impose challenges in some limited resources settings in preserving the quality and quantity of DNA. In this mini review, we summarise the research findings on different minimally invasive sampling techniques for collecting quality DNA samples appropriate for telomere length measurement study.

METHODS

The literatures were searched in Scopus, PubMed and google scholar databases using these keywords: telomere length AND qPCR AND (Blood OR saliva) AND human until Jan 2022. Then, the title and abstract of each article were read and screened for relevancy. Articles were selected following these inclusion criteria: 1) published between Jan 2011- July 2021; and 2) studies related to telomere measurement using qPCR on human samples 3) published research article. Meanwhile, animal and cell-line studies were excluded. Finally, seventeen research articles were narrowed, selected, and reviewed for this paper.

RESULT AND DISCUSSION

Minimally Invasive Sources for Quality and Quantity of DNA

Minimally invasive sampling method refers to a procedure that can give minimal risk to the body, such as the procedure used to collect venous blood, DBS, saliva, and urine. To measure the telomere length using qPCR, a good DNA is needed as a base for the amplification step. However, different sample types have different yields of genomic DNA due to the varied properties of cells of individuals. After sample collection, genomic DNA needs to be extracted and quantified for its quantity and purity as quality control checking. Using spectrophotometry method, DNA purity ranges from 1.8-2.0 of 260/280 ratio is considered ideal (13). A ratio of <1.8 or >2.1 is indicative of protein or salt contaminations (14). Suggested that DNA purity (260/280) is correlated with contamination during extraction which influences the qPCR reaction (15). The stability of DNA quality must be ensured where degraded or fragmented DNA could be evaluated by running the DNA using gel electrophoresis. An observation of a clear band of DNA without severe smearing of DNA banding generally marks the quality of DNA. In addition to quality, different types of DNA samples have been observed to yield different quantities of DNA (concentration). Based on the seventeen articles reviewed, we determined the quality and quantity of DNA obtained from different samples obtained by minimally and non-invasive collection methods, namely venous blood, dried blood spot (DBS), and saliva for telomere length measurement. We found that eight studies used venous blood, five studies used dried blood spot and four studies used saliva for sample collection Figure 1. A detailed overview of DNA concentration between these sample types is summarised in Table I.

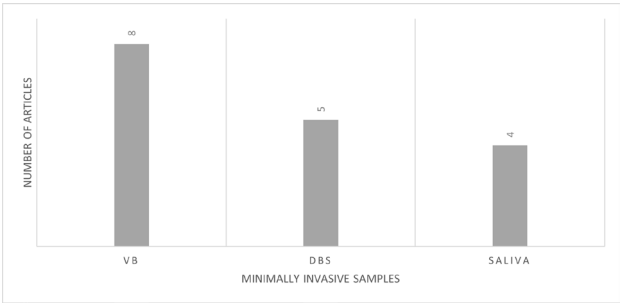

Figure 1: Source of samples among sixteen articles. Seven articles use VB (11,16–19,21–23) as their sample of choice, five articles use DBS (11,27–30) and four articles use saliva (11,29,32,33) for TL measurement using qPCR

Table I: Mean DNA concentration of different sample types

Parameters and Measurement	Sample Type	Mean DNA concentration (ng/μL)	Sample size	Result	Reference
Telomere lengt: qPCR	VB	>100	2006 individuals	There was an association between the consumption of Chinese tea and TL in men	(16)
				Shortest telomere was found in individuals who consume a high amount of Chinese tea (>3 cups/day)	
Telomere length: qPCR	VB	N/A	155 nurses	nurses in night shift workers have longer TL due to younger age, shorter working employment hour and physical activity.	(17)
				TL was a reliable indicator for a biological marker for ageing	
Telomere length: qPCR	VB	20	356 children	Shorter telomere was associated with high blood lead concentration among children	(18)

Table I: Mean DNA concentration of different sample types (CONT.)

Parameters and Measurement	Sample Type	Mean DNA concentration (ng/µL)	Sample size	Result	Reference
Telomere length: qPCR	VB	N/A	2307	There was a significant association between environmental exposure to urinary antimony and shorter TL.	(21)
Telomere length: Monochrome Multiplex qPCR	Finger DBS	1.84	40 females	DNA yield too low for TL measurement for DBS	(11)
	Vein DBS	1.39			
	Saliva	Oragene: 57.7		T/S ratio between buffy coat, Origen Saliva and Oasis Saliva were significantly different	
		Oasis: 12.5			
Telomere length: qPCR	DBS	56	95 African American men	No association between racial discrimination or implicit racial bias on TL	(27)
Telomere length: Multiplex qPCR	DBS	530.34	19 individuals from the University of Washington	TL measurement moderately correlated between DBS and WB	(28)
		397.33	15 individuals from Emory University	TL significantly different under different DBS condition	
Telomere length: qPCR	DBS	59.1	24 individuals	TL highly correlated between DBS and WB	(29)
				Salivary TL strongly correlated DBS	
				Salivary TL correlated with WB TL	
	WB	N/A		Salivary TL was also correlated with whole	
	Saliva	6.03 µg		TL was longer in saliva than in whole blood or DBS	
Telomere length: qPCR, multiplex qPCR	DBS	N/A	12 individuals	T/ S ratios from DBS were higher than PBMC and WB	(30)
Telomere length: qPCR	Saliva		200 students	There was no significant TL shortening in response to AS in adolescent students facing their final examinations	(32)
Telomere length: qPCR	Saliva	N/A	40 children	There was significant correlated between saliva and blood leukocyte	(33)
			200 students	Shorter telomere is associated in disadvantaged environment group	

N/A: Not applicable, VB : Venous blood, DBS: Dried blood spot, WB: Whole blood, PBMC: Peripheral blood mononuclear cell, TL: Telomere length

Venous Blood

Venous blood (VB) obtained by direct puncture into a vein in the antecubital area of the arm or at the back of the hand. Researchers (16–19) use VB as it is considered as a gold standard sample for telomere assay as it contains abundant and stable DNA. In order to retain the DNA integrity, blood needs to be transported in ice and extracted immediately or stored after collection. From venous blood itself, researcher needs to decide which blood components would be the best sample of choice for good quality and quantity of DNA ranging either from the whole blood (WB), peripheral blood leukocyte, buffy coat, or serum. Ethylenediaminetetraacetic acid (EDTA) tube is commonly used for the whole blood sampling, while plain tube/serum separating tube (SST) is used for buffy coat or serum. Acid citrate dextrose (ACD) tube also can be used to collect the whole blood, buffy coat, and all cell pellet (ACP) (14,20). Comparison study of DNA yielding (20) has been reported that the whole blood yielded more DNA than buffy coat from fresh samples with 89.9 µg/5mL and 40.40 µg/5mL, respectively (20) (Table I). This finding is supported by another study where (14) 318 µg/mL for whole blood and 252.09 µg/mL for buffy coat (14). Recent telomere measurement studies (21–24) also used whole blood as their sample source.

Prolong storage of blood may affect the DNA integrity. Storing samples at appropriate temperature is crucial to avoid any blood proteins or enzymes, released through cell lysis, from denaturing the DNA (25). A study showed there was significant decrease of DNA yielding by ~40% (WB) and 70% (buffy coat) by PicoGreen and Nanodrop analyses after being stored for a year at -80°C (14). Another study showed that blood stored at 4°C for 2.5 years had a clear DNA banding with minimal smearing on agarose gel indicating that the DNA was not degraded or fragmented (20). Thus, it is advisable to process the sample as soon as possible after the blood is collected. Although the collection of VB gives a high yield of DNA, there are several drawbacks for using this technique such as pain at the injection site, requirement of a phlebotomist, and storage of blood in the freezer is

required to maintain DNA integrity for a longer period of time.

Dried Blood Spot

Dried blood spots (DBS) are referred as finger prick blood that is spotted onto specialised filter paper (Whatman 903 or FTA card) using single use lancet then dried afterwards (26). Studies used dried blood spot (DBS) for telomere measurement as it is effective and provides promising results (11,27–30). Telomere length in DBS was the closest approximate with that of the whole blood as DBS sample have the same cell composition with the vein whole blood (VWB) in terms of haemoglobin, white blood cell and platelets (28).

In terms of DNA yielding, theoretically DBS has a low DNA concentration due to the amount of blood spotted (~50µL) on card using finger prick lancet. However, the use of DBS resulted in an insufficient amount of DNA (11) to measure telomere length, despite using a modified protocol for the MMQPCR assay (Invitrogen Charge Switch Extraction kit) was used and the elution volume for DNA was reduced to 60µL. In a comparison study between WB and DBS, reported that T/S ratio of DBS is significantly higher than WB blood suggesting that telomere length could be affected by sample collection methods (30). Suggested that this could happen due to the composition of cell subtypes residing in the DNA of WB and DBS that may contribute to the discrepancy of the telomere measurement. Finger capillary blood have large granulocyte count than VB which contribute to high T/S ratio (30). Previous studies reported that DNA yield in DBS was too low (11) to perform reliable telomere measurement. Therefore, the validated DBS telomere measurement using QIAamp DNA Investigator Kit extraction has a higher DNA yield (530.34 ng/µL) (28). This finding is also supported by another study (20) which obtained an average DNA yield of 54 ng/µL from DBS.

On the other hand, shipping and storing of DBS also need to take into consideration. Most of researchers (11,27–30) just dry the DBS at room temperature (RT), then stored at -80°C before extraction. A study (28) that examined various conditions that can occur during sample transport and export to the laboratory found that a partial extraction protocol (frozen at -30°C and shipped in dry ice, then partially extracted and stored for 7 days at RT) provided reliable T/S measurements if researchers encountered regulatory difficulties during the export process. Despite yielding acceptable quantity and quality of DNA, DBS also is suitable in a limited resources place. The low cost of capillary DBS collection and the ease with which DBS samples can be collected, transported, and stored, particularly in field settings, have resulted in the widespread use of this sampling technique in an epidemiological study. Unlike VB, DBS does not require a phlebotomist and only requires minimal amount of blood (~50 ul) and less invasive method (needle prick on fingers) compared to VWB (21).

Saliva

In non-clinical settings, saliva is suitable for sample collection due to its simple procurement and applicable for almost all targeted individuals, especially children. Saliva collection is effortless compared to blood that requires professional skills and may be invasive and painful, resulting in low compliance for donors to donate their samples. Although saliva contains epithelial cells and leukocytes, other studies (31,32) reported that TL measurements using saliva were reliable and found a significant correlation between TL in saliva and TL in leukocytes (33). Shorter telomere were also found in children raised in disadvantaged families (33) and children with high genetic sensitivity (27). However, a study reported no association between academic stress and telomere length in adolescents (32). Another study validated the use of saliva as effective and applicable for TL measurement (29). In addition, saliva also provided higher DNA concentration (2.48-308.0 ng/µL) than buffy coat (0.82-125.0 ng/µL) (11) (Table I).

Four researchers (11,29,32,33) collected saliva using Oragene Saliva Kit (Oragene® by DNA GenotekTM), an established kit that permits collected samples to be stored at room temperature up to 5 years. To protect the integrity of DNA, this saliva self-collection kit contains reagents to inhibit degradation and prevent bacterial contamination (34) which previously was the primary concern among researchers (35). Purchase of saliva kits may incur an additional cost, but fees for collecting blood samples and laboratory services can be waived. Despite the price, saliva is a good alternative source that involves non-invasive procedures. It offers a simple procurement and is suitable for individuals who have anxiety or trypanophobia (afraid of needle).

LIMITATION OF STUDY

In the interpretation of our review, several limitations will be kept in mind. Only a few studies evaluated the minimally invasive technique with TL measurement and cross tissue comparison between different sources of samples. Some of the researchers do not detail the yielding and purity of DNA. Thus, it was hard to compare the mean DNA yielding and purity among articles.

CONCLUSION

There are several factors that researchers need to consider before deciding on the source of DNA for the measurement of TL. Procuring DNA samples via a minimally invasive procedure that provides an acceptable quantity and quality of DNA is favourable. However, other factors should also be considered in deciding the best option of sample sources for measuring

TL. Factors such as the research settings and facilities and the sample population are worth considering in addition to having samples with good quality and quantity of DNA suitable for measuring TL. In this review, we highlight the comparison of DNA quality and quantity of various DNA samples obtained from minimally invasive procedures, namely VB, DBS, and saliva. It was found DNA concentration varies in different sample types with different volume of sample collected. VB was found to have a higher DNA yield, followed by saliva and DBS. VB is also stable to be stored at -80°C for a longer period before extraction. On the other hand, saliva test kit was deemed suitable for children respondents especially in limited resourced settings. Saliva can last longer, up to 5 years at room temperature compared with blood which require storage in -20°C and -80°C for DBS and VB respectively.

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