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The Quality of mt-loop DNA Segment of Smokers and Nonsmokers in Lagos, Nigeria for Possible Use in Forensic Biology

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Abstract. Mitochondrial DNA (mtDNA) is a small circular DNA responsible for transmission of traits. In forensic biology, the high sensitivity of mtDNA analysis allows forensic scientists to obtain information from evidence associated with crime scene. This study was carried out to investigate the mtDNA segment of smokers and non-smokers and to determine to what extent smoking affects the quality of the mtDNA in the sample population. Twenty five cigarette butts were obtained from a bar and twenty five samples were also obtained from saliva of non-smokers using swab stick. Mitochondrial DNA (mtDNA) was extracted from individual samples using zymo kit, spectrophotometer was used to check for the concentration and purity of the extracted mtDNA. Polymerase chain reaction (PCR) was carried out in a gradient thermocycler to ascertain the hypervariable region of the mtDNA using the following primer sequence. Agarose gel electrophoresis was carried out to know the amplicon size using 100 base pair of ladder. The DNA purity on saliva extract for non-smokers was found to be higher ($A_{260/280}$ 2.06 - 1.82) than the purity of saliva from smokers ($A_{260/280}$ 1.82-1.0). The concentration of DNA found on the saliva traces from non-smokers was higher (26.2 - 3.0 ng/µl) than those extracts from smokers (26.2 - 2.23ng/µl). DNA bands obtained from agarose gel electrophoresis showed amplification of the hypervariable region of mtDNA size ranges from 295-300 base pair (bp). This study showed that the hypervariable region of the mtDNA of both smokers and non-smokers have the same range of nucleotide base pair.

Keywords: mtDNA; Saliva; Cigarette; Smoker.

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1. Introduction

Forensic identification is an effort to help law enforcement in determining a person's identity. Personal identity is often a problem in criminal cases, civil cases, death without identity, and mass disasters[1]. Personal identification is defined as the act of establishing the identity of an individual. It arises in natural mass disasters like earth quakes, tsunamis, landslides, floods etc., and in man-made disasters such as terrorist attacks, bomb blasts, mass murders, and in cases when the body is highly decomposed or dismembered to deliberately conceal the identity of the individual[2].

There are several types of personal identification in forensic biology such as DNA analysis, Friction Ridge Analysis, Forensic Odontology, Biometric detection[3,4]. Deoxyribonucleic acid(DNA) analysis of evidential material is a powerful tool for linking an individual to a victim or a crime scene, DNA is a molecule that carries genetic material[5] which has been successfully extracted from many biological sources such as blood, semen, saliva, skin cells and used for different scientific analysis[6].

Human DNA is found in every cell except erythrocytes[7,8]. DNA is unique to every individual and DNA typing methodologies are continuously subjected to scientific analysis. It is important because it contains genetic information which can be used in disease detection, profiling the paternity of a child, examining the biological mother of a child[9] and to identify individuals by the characteristics of their DNA for resolution of criminal cases.

The human genome contains about 3 billion base pair which is important in carrying genetic information that helps to identify an individual[10]. In human cells, most DNA is found in a compartment within the cell called a nucleus and it is known as nuclear DNA[11]. In addition to nuclear DNA, a small amount of DNA in humans and other complex organisms can also be found in the mitochondria, this DNA is called mitochondrial DNA (mtDNA). Most plants have a set of DNA contained within their chloroplasts called chloroplast DNA (cpDNA), the complete set of DNA is known as the genome[12]. Hypervariable region (HVR) of the mtDNA is a location within nuclear DNA or the D-loop of mitochondrial DNA in which base pairs of nucleotides repeat (in the case of nuclear DNA) or have substitutions (in the case of mitochondrial DNA) (Mannis and Manfred, 2008). mtDNA hypervariable region are divided into three types:

Hypervariable region 1 (HVR-I), Hypervariable Region 2 (HVR-II) and Hypervariable Region 3 (HVR-III). HVR1 is considered a low resolution region and HVR2 is considered a high resolution

Changes or repeats in the hypervariable region are highly polymorphic and these regions are useful in human mitochondrial genealogical DNA testing and widely used as a tool in many fields including evolutionary anthropology and population history, medical genetics, genetic genealogy, and forensic science.

Maternal inheritance of mtDNA allow scientists to compare the mtDNA profile of a set of remains to that of reference samples from individuals such as the mother, brother(s), sister(s) or any other maternal related individuals of a missing person. These samples should have the same mtDNA profiles because all maternal relatives inherit the same mtDNA. Since mtDNA is maternally inherited and multiple individuals can have the same mtDNA type, unique identifications are not possible using mtDNA analyses. However, mtDNA is an excellent technique to use for obtaining information in cases where nuclear DNA analysis is not feasible.

It is generally accepted that about 10% of genome is genetically relevant and the other non-coding region, which represents about 90% of the human genome, has part of it as repetitive sequence[13]. The repetitive sequence forms number of blocks that varies considerably among unrelated individual. These repetitive sequences are majorly categorized into mini and micro satellites[14,15]. Satellite repeats possess extreme diversity in their monomer size, nucleotide sequence, complexity, genomic distribution, and abundance even in closely related species[17,18]. Microsatellites are widely used for DNA profiling (genetic fingerprinting) in forensics biology such as in crime scene analysis and also in tissues transplant in patients. They are also widely used in kinship analysis (most commonly in paternity testing).[18] These microsatellites includes short tandem repeats (STRs), Simple Sequence Length Polymorphisms (SSLP), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), random amplification of polymorphic DNA (RAPD) etc[19].

DNA has been used as a unique investigation material for forensic purposes[8]. DNA amplification methods have been developed to quantify DNA since mid-1980s[20]. This method is referred to polymerase chain reaction (PCR).

The methodology of DNA typing is majorly coupled with the sensitive polymerase chain reaction, and it has ever since been used for research based analysis and legal scrutiny individualization.

Every individual has a distinct DNA and methodologies of DNA typing are continually exposed to scientific and legal scrutiny[21]. DNA traces can be found in body fluid; like blood, saliva, teeth, semen, vaginal secretion, bones, hair and perspiration[22]. DNA Forensic samples can be composed of DNA from more than one individual, making the typing results more complex[23]. Forensic materials that contain degraded DNA or minute amount of DNA may not yield a STR result[24].

The degradation could be as a result of environmental conditions, hence the need to investigate the quality and purity of DNA; cigarette butt remains a very quality source where we can get DNA because of the dried saliva of the smoker on it. DNA samples recovered from dried saliva in a cigarette butt left at crime scene are frequently exposed to detrimental environmental factors (such as light, heat, decomposition of bacteria among others) before they are collected, which will affect the results of the analysis that will be carried out on them[25].

The possibility of obtaining a reliable sample that could be analysed as evidence becomes a huge challenge to the forensic odonatologists[26]. Traces of saliva have been known to be mainly found at cigarette butt which takes 18 months to 10 years to decompose, depending on condition. These traces of saliva from the cigarettes butts are useful in forensic practice[25].

Currently, about 1.2 billion people globally smoke tobacco whenever they are distressed and some other times get addicted. The number of smokers worldwide has increased to 1.1 billion in 2019, with tobacco smoking causing 7.7 million deaths including 1 in 5 deaths in males worldwide[27].

In Nigeria, about 16100 tobacco-related deaths occur annually[28]. It is likely that these numbers may be grossly underestimated because of weak surveillance systems. In addition, 5.6% (4.7 million) of Nigerian adults currently use a tobacco product and 3.9% (3.1 million) adults are current tobacco smokers[29]. Of greater concern is tobacco smoking by children and adolescents where 25000 Nigerian children (aged 10–14 years) smoke cigarettes each day. Cigarettes are affordable for young people in Nigeria because they are still being

sold in single sticks, despite the provision of Article 16 of the Framework Convention on Tobacco Control to which Nigeria is a signatory[30,31].

In forensic practice, the most common carriers of saliva traces are cigarette butts, chewing gums, postage stamps and envelopes. Saliva is a complex biological fluid secreted by acinar cells of the major and minor salivary glands. It is an indicator of various plasma constituents. In recent years, its role as a diagnostic and forensic tool is being increasingly researched upon and evaluated. Besides maintaining the homeostasis of oral structures such as tooth integrity. It also plays a critical role in genomics, proteomics, metabolomics, and bioinformatics. It is an important discriminating element in forensic biology, acting as an indicator of salivary gland conditions and toxicological and drug monitoring[32,33,34].

In a crime scene, smokers leave their cigarette butts unknowingly, sometimes could help forensic scientists to clarify identities of individuals involved in the crime by extraction of the DNA in the leftover saliva. In other words, these could lead to misinterpretation of the events of the crime scene and compromise the results from the event. Also there are some methods of DNA extraction for trace DNA such as from butts but their DNA yield and quality were not as good to get a good genotyping results. Examination of saliva traces left on cigarette butts as evidences are complicated due to the availability of the biological material in trace amounts for analysis and its rapid degradation due to extreme effects of environmental factors. There is a growing need to perform trace analyses such as DNA salivary analyses on cigarette butts found at a crime scene for identification purpose. Hence, this study will compare the DNA quality and quantity in cigarette butts recovered from smokers and analyzed with specific primers to determine its yield and quality. The positivity of the result will determine its use in forensic studies in Nigeria.

The aim of this study is to assess the quality of MT-loop DNA segment of smokers and nonsmokers for possible use in forensic biology. The specific objectives of this study were to determine the quantity and quality of DNA extracted from cigarette butts and swab stick, determine the concentration and purity of DNA using Nano-drop Spectrophotometer and evaluate the amplification of mt-DNA of smokers and nonsmokers using PCR and primer.

2. Methodology

2.1. Sample collection

Twenty-five samples of used cigarette butts were provided by smokers from a bar at University Road Lagos Mainland Akoka, Yaba, Lagos and twenty-five samples were collected from buccal cells of non-smokers (students) from faculty of Science, University of Lagos using swab stick. The samples were aseptically transferred into zipped poly and red cap bottles then stored at -20^{o}C till further experiments.

2.2 Ethics approval

Application for ethics approval for the research was obtained. The Ethical Approval Number is CMUL HREC, Registration Number: NHREC/19/08/2019B.

2.3 DNA extraction

Each of the twenty-five (25) butts was cut in Eppendorf tube containing 500ul of genomic lysis buffer. The samples were vortexed for 4–6 secs and centrifuged at 10, 000 rpm for one (1) minute to dislodge other materials. It was allowed to stand at room temperature for 5–10 minutes. The suspension was transferred to a Zymo-Spin^{TM} column in a collection tube and centrifuged for a minute. The residue was discarded from the flow through and the supernatant in the Zymo-Spin^{TM} column was transferred to a new collection tube. DNA prewash buffer of about 200ul was added to the spin column and then centrifuged for a minute. Following this, 500ul of genomic DNA wash buffer was added to the spin column and centrifuged again for another one (1) minute. The suspension in the spin column was transferred to a clean micro centrifuge tube and 100ul DNA elution buffer was added to it. This mixture was incubated for 2–5 minutes at room temperature. It was then centrifuged at 12000rpm for 30 seconds to elute the DNA.

2.4 DNA quantitative analysis

The DNA quantity was measured using the Nano-drop spectrophotometric analyzer with a wavelength of 260 and 280. The DNA quantity of smokers' ranges from 3.0-56.7ng/μl and purity level ranges from 1.0-1.82 while that of the non-smokers ranges from 2.23-52.6ng/μl and the purity level ranges from 1.14-

2.06 respectively. The absorbance quotient (OD_{260}/OD_{280}) provides an estimate of DNA purity. An absorbance quotient ratio < 2.0 was considered to be purified DNA. A ratio of < 1.8 is indicative of protein contamination, whereas a ratio > 2.0 indicates DNA contamination.

2.5 Agarose gel electrophoresis

The integrity of genomic DNA was tested by resolving DNA extract on a 1% agarose gel by electrophoresis followed by visualization with 8ul ethidium bromide staining. Each DNA sample was graded according to the electrophoretic migration of sample DNA compared with a known molecular weight marker[35]. PCR amplification products (10 µl) were subjected to electrophoresis (Bio-Rad) on 1% agarose gel in 0.5× Tris-Borate-EDTA buffer at 70 V for 1hr and stained with ethidium bromide (Himedia), and images were obtained in gel documentation (G-Box; Syngene, Cambridge, UK) systems.

2.6 Polymerase chain reaction (PCR)

DNA extracts for the PCR-based assays was assessed by amplifying the mtDNA D-loop region, which was amplified by PCR using primers forHV1 and HV2 region ofmtDNA which are: Hyper-variable Region 1A (HV1A)Primer A1(L15997)-5`CAC CAT TAG CAC CCA AAG CT 3` and Primer B2(H16236)-5` CTT TGG AGT TGC AGT TGA TG 3`.PCR was carried out in 550µl total reaction volume, each containing 200ng template DNA, 0.5uM of each primer in 5x master mix of $MgCl_2$,dNTPs, and Taq DNA polymerase. The reaction mixture was heated under the following thermal profile: Initial Denaturation at 94°C for 5 mins, final denaturation of 94^oC for 1min, followed by 40 cycles; annealing at 53°C for 1min, extension at 72°C for 1.5min, and final extension at 72^oC for 10mins, and final holding at 80^0Cuntil sample removed from thermocycler. The assembled reaction was sealed, vortexed, centrifuge and placed in the thermocycler for DNA amplification. The amplification was carried out in Prime Thermocycler in Botany Department, University of Lagos.

2.7 SPSS statistical analysis

Statistical analysis was by one way analysis of variance (ANOVA) using SPSS 20.0 version for the analysis on DNA isolation, P value lesser than (<) 0.05 was considered significant.

3. Results

The results from this study showed that the purity and concentration of DNA extracted from saliva traces on smoked cigarette butt and swab stick of non-smoked to have variation among various sample as shown in Table 1 and 2 below.

Table 1 shows the values of the means and standard deviations for purity of DNA obtained from saliva traces on cigarette butts and swab stick of non-smokers. The purity absorbance range of sample 6 non-smoker group with the ratio of A260/280 was the highest with mean and standard deviation of 2.06 ng/ul ± 0.62 ng/ul, and the highest purity absorbance range of A260/280 ratio of DNA obtained from saliva traces on the cigarette butts were 1.87 ng/ul ± 0.42 ng/ul in sample number 1 of the smokers group. However, sample number 14 showed the lowest purity absorbance range of A260/280 ratio of DNA with 1.06 ng/ul ± 0.07 ng/ul among the smokers, while sample number 13 showed the lowest purity absorbance ratio of A260/280 of DNA with 1.14 ng/ul ± 0.07 ng/ul.

Table 2 shows values of the means and standard deviations for the concentration of DNA obtained from saliva traces on cigarette butts and swab stick of non-smokers. It was observed that the concentration level of DNA obtained from saliva traces on swab stick in sample no. 5 of non-smoker group was the highest with means and standard deviation of 52.64 ng/ul ± 10.31 ng/ul, and the highest concentration level of DNA obtained from saliva traces on the cigarette butts were 56.67 ng/ul ± 63.88 ng/ul for sample number 1 of the smokers group. However, sample number 1 presented the lowest concentration level of DNA with 2.37 ng/ul ± 1.55 ng/ul in the smokers group, while sample number 21 showed the lowest concentration level of DNA with 2.23 ng/ul ± 0.48 ng/ul for the non-smokers.

Table 1. Purity of DNA from smoked cigarette butts of smokers and swab stick of non-smokers using spectrophotometer.

SAMPLE TYPE (SMOKER)	PURITY $A_{260/280}$ ± SD	SAMPLE TYPE (NON-SMOKER)	PURITY $A_{260/280}$±SD
S1	1.87 ± 0.42	NS1	1.93±0.38
S2	1.43 ± 0.27	NS2	1.69±0.18
S3	1.25 ± 0.10	NS3	1.59±0.28
S4	1.39 ± 0.34	NS4	1.61±0.25
S5	1.37 ± 0.17	NS5	1.61±0.16
S6	1.78 ± 0.58	NS6	2.06±0.62
S7	1.21 ± 0.12	NS7	1.87±0.09
S8	1.14 ± 0.16	NS8	1.46±0.45
S9	1.58 ± 0.31	NS9	1.36±0.16
S10	1.13 ± 0.03	NS10	1.53±0.23
S11	1.25 ± 0.04	NS11	1.34±0.16
S12	1.41 ± 0.32	NS12	1.41±0.32
S13	1.23 ± 0.04	NS13	1.14±0.07
S14	1.06 ± 0.07	NS14	1.72±0.28
S15	1.26 ± 0.11	NS15	1.63±0.33
S16	1.52 ± 0.08	NS16	1.40±0.07
S17	1.19 ± 0.02	NS17	1.53±0.32
S18	1.18 ± 0.04	NS18	1.97±0.03
S19	1.21 ± 0.11	NS19	1.29±0.20
S20	1.14 ± 0.01	NS20	1.90±0.09
S21	1.30 ± 0.13	NS21	1.79±0.16
S22	1.11 ± 0.01	NS22	1.61±0.18
S23	1.18 ± 0.03	NS23	1.33 ± 0.26
S24	1.25 ± 0.04	NS24	1.66 ± 0.31
S25	0.98 ± 0.15	NS25	1.74 ± 0.10

3.1 Agarose gel electrophoresis for genomic DNA after extraction from cigarette butts and swab stick

Figure 1 shows the migration of genomic DNA band from negative anode to positive cathode in agarose gel electrophoresis. However, it was observed that some genomic DNA samples were stuck in the well.

Table 2. Concentration of DNA from smoked cigarette butts of smokers and swab stick of non-smokers using spectrophotometer.

SAMPLE TYPE (SMOKER)	CONCENTRATION (ng/ul) ± SD	SAMPLE TYPE (NON-SMOKER)	CONCENTRATION (ng/ul) ± SD
S1	2.37 ± 1.55	NS1	34.40 ± 7.00
S2	5.6 ± 3.17	NS2	18.21 ± 2.09
S3	9.97 ± 4.99	NS3	32.74 ± 9.56
S4	6.60 ± 5.47	NS4	21.80 ± 1.87
S5	8.73 ± 8.40	NS5	52.64 ± 10.31
S6	11.60 ± 14.91	NS6	19.30 ± 7.88
S7	12.20 ± 14.89	NS7	19.81 ± 8.16
S8	22.30 ± 19.29	NS8	35.32 ± 8.30
S9	3.77 ± 2.73	NS9	13.42 ± 3.25
S10	5.40 ± 1.39	NS10	30.00 ± 1.99
S11	15.43 ± 9.57	NS11	23.00 ± 2.69
S12	9.27 ± 7.40	NS12	12.50 ± 3.23
S13	6.90 ± 2.29	NS13	6.02 ± 1.16
S14	27.07 ± 32.12	NS14	15.40 ± 4.73
S15	22.90 ± 24.86	NS15	10.61 ± 2.96
S16	9.67 ± 4.90	NS16	9.80 ± 4.62
S17	16.23 ± 14.89	NS17	7.00 ± 0.98
S18	56.67 ± 63.88	NS18	8.03 ± 0.78
S19	9.27 ± 3.23	NS19	15.61 ± 2.30
S20	10.67 ± 2.32	NS20	29.60 ± 5.35
S21	25.20 ± 30.81	NS21	2.23 ± 0.48
S22	6.87 ± 0.90	NS22	2.66 ± 0.65
S23	6.83 ± 6.40	NS23	10.80 ± 2.71
S24	10.00 ± 4.75	NS24	14.70 ± 2.55
S25	24.13 ± 2.59	NS25	2.33 ± 0.85

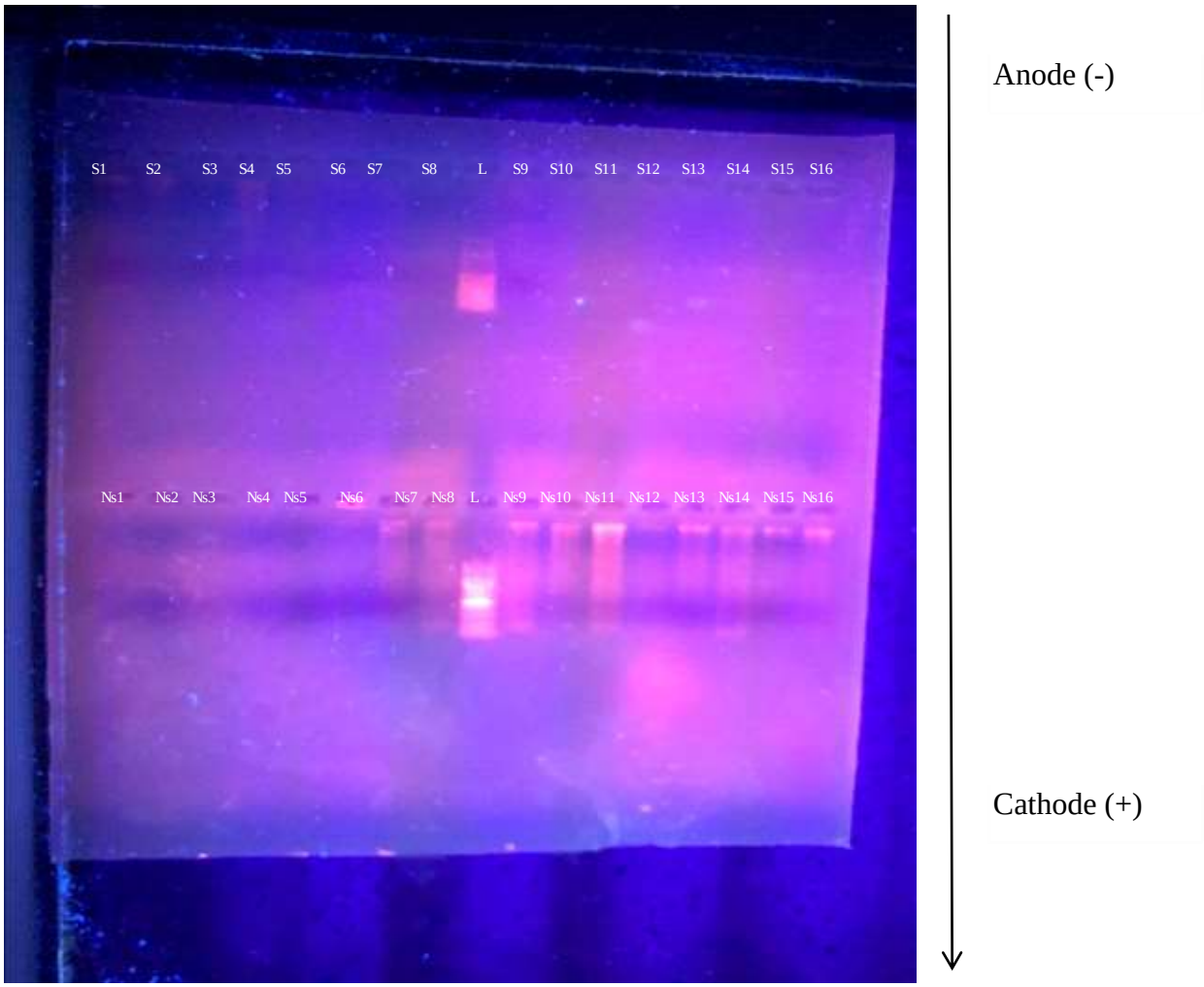

Figure 1. The Presence of DNA after extraction from swab sticks.

3.2 Agarose gel electrophoresis after polymerase chain reaction (PCR)

Figure 2 shows the amplification of hypervariable region of mtDNA after polymerase chain reaction indicating the amplicon size is 295-300 base pair (bp) agarose gel electrophoresis. The amplicons migrated from negative to positive corresponding to themolecular weight marker (L) of 100 base pair (bp) in the gel. However, sample N2, N3, N7, N9, N10, N11, N12, N14-N17, N19, N22-N23 and N25 among non-smokers group and sample S9-10, S12, S15 and S21-25 among the smokers group were not amplified.

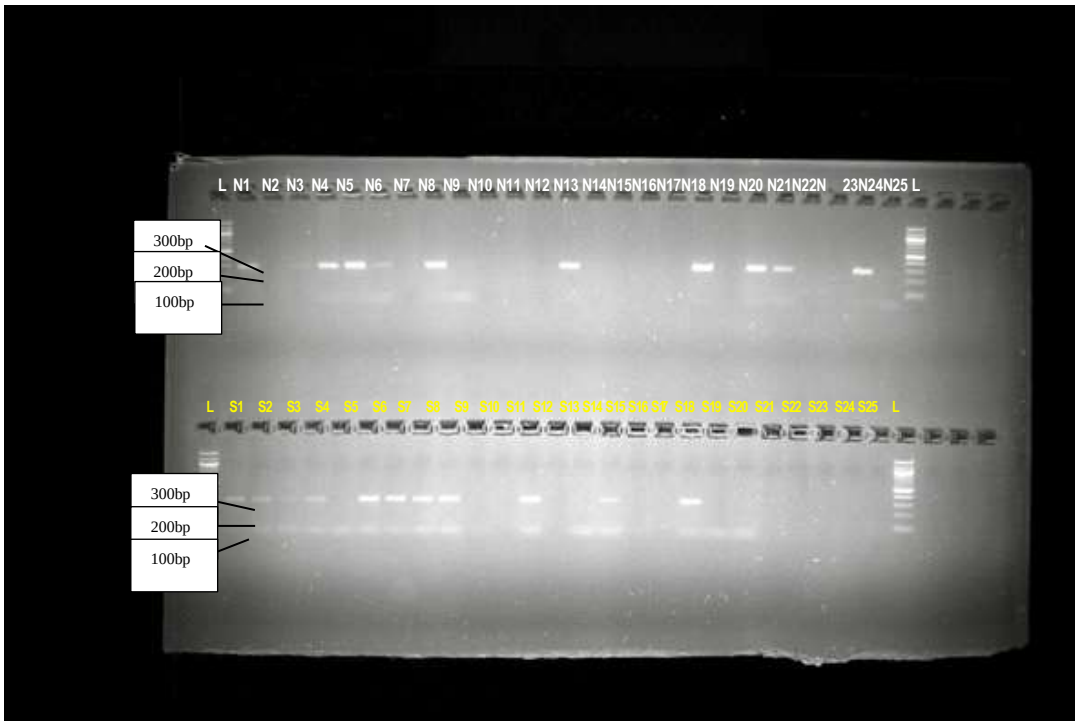

Figure 2. Amplification of hypervariable region of mitochondrial DNA (mtDNA) between smokers and non-smokers.

4. Discussion

Saliva is a complex biological fluid secreted by the acinar cells of the major and minor salivary glands. It is an indicator of various plasma constituents. In recent years, its role as a diagnostic and forensic tool is being increasingly researched upon and evaluated[36]. It is an important discriminating element in forensic biology as the whole saliva is a mixture of secretions released from salivary glands and gingival crevicular fluid, also contains exfoliated buccal epithelial cells and microorganism[37]. It acts as an indicator of salivary gland conditions, toxicological and drug monitoring[32]. Buccal cell DNA quality is of great importance in solving and investigating events in forensic science[25]. Buccal cell's DNA is often found in the saliva and is a common biological evidence in forensic science. The examination of saliva traces left on cigarette butts as evidences are complicated due to the availability of biological material in trace amounts and its rapid degradation due to extreme effects of environmental factors.

In forensics, Mitochondrial DNA (mtDNA) is an extra nuclear genome which has certain features that makes it desirable for forensic scientist. These features include high copy number, lack of recombination and matrilineal

inheritance. mtDNA typing has become routine in forensic biology and it is used to analyze old bones, cheek cells, teeth, hair shafts and other biological samples where nuclear DNA content is low[38].

In this study, the purity of DNA obtained from non-smoker group showed higher average absorbance range of A260/280 ratio of 2.06 ng/ul ±0.62 ng/ul than the purity of DNA obtained from the smoker group, which presented absorbance range of A260/280 ratio of 1.06 ng/ul ±0.07 ng/ul. This may indicate the environmental exposure to high temperatures, which may have influenced the degradation of DNA in saliva deposited on the cigarette butts. It is consistent with the report by Bonanomi *et al.*[39], in which the authors concluded that the decomposition in cigarette butts is mainly controlled by temperature, water availability, and biochemical quality in terms of organic types of nicotine content. Another factor that may result in the low purity level of DNA is related to the fact that chronic smokers hardly deposit large amount of saliva on cigarette butts when smoking. It may also suggest that the presence of carbohydrates, magnetic beads, residual phenol or proteins, which is consistent with the fact the cigarette butt filter paper is made up of cellulose acetate with isa plant origin – which interferes on the purity of the extracted DNA[40]. Peptides, carbohydrates, aromatic compounds, buffer salts, and extracellular proteins like mucus in saliva can contaminate and damage DNA integrity, causing the purity of the DNA extract to fall below the optimal range[41].

The results in the present study showed that the concentration of the DNA extracted from cigarette butts was higher, with average of 56.67 ng/ul ± 63.88 ng/ul, in the smoker group compared to the concentration of the DNA extracted from swab stick for the non-smoker group, with average of 52.64 ng/ul±10.31 ng/ul. This result contrasts what was reported by Casey[42]. This might suggest that the extraction kit and the protocol was better when compared to that of Casey[42].

What was observed in the agarose gel electrophoresis for genomic DNA indicates that there was DNA in the extracted samples, and the bands showed that the DNA was good enough and of high quality. The research then proceeded to the next level of the analysis using the polymerase chain reaction (PCR). The results obtained showed the amplification of mtDNA. The band seen in the region of 295-300 base pair corresponding to molecular weight

marker (l) of 100 base pair among non-smokers and smokers suggests that the mtDNA was amplified and it could be of relevance for forensic analysis. mtDNA has been shown to be more durable than nuclear DNA (nDNA) using electron microscopy. The results are attributed to its high number of copies per cell and the tolerance to extreme environmental circumstances, as well as other unique characteristics such as haploid maternal inheritance, high levels of variety, absence of recombination, lack of introns and histones and due to rapid evolution[43]. This study also found that both smokers and non-smokers have the same nucleotide base pair (bp) ranges in the amplified hypervariable region of their mtDNA.

5. Conclusions

This study was carried out to determine the reliability of mtDNA in forensic biology and the effect of smoking on this reliability. The results of the initial extraction showed that DNA purities of non-smokers were higher than those of smokers, suggesting overall negative effects of smoking on DNA purity. However, this had no effect on the amplifications of the mtDNA during PCR analysis since the DNA of both smokers and non-smokers showed amplification at almost the same range of base pair (bp). The findings of the study showed clearly that smoking had no discernable effect on the stability of mtDNA amplification. This suggests that environmental influences have no effect on mtDNA function. This study revealed that the amplified region of mtDNA can be used for various forensic studies. However, more research at the genomic level is needed to understand the overall variation in the region by sequencing it, which will aid in a precise identification of an individual.

References

1. Yudianto A, Masniari N, Muhamma AJ, Deka BB. DNA Methylation on Bloodstain as a Forensic Age Estimation Method. Proceedings of the International Conference on Law, Economics and Health (ICLEH 2020). 2020;52 (1):1863-8. https://doi.org/10.2991/aebmr.k.200513.006
2. Modi JP. A Textbook of Medical Jurisprudence and Toxicology. 24th Ed. W. Wood and Company. 2011.

3. Tanuj K, Kewal K. Personal Identification in Forensic Examinations. Anthropol. 2013;2 (1):1-2.
4. Oday AH, Nur AA, Zainal ZA. Human Identification System: A Review. Intern Journ of Comp & Bus Res. 2019;9(3):20-7.
5. Falch M, El-Sayed MJS, Takousis P, Pesce F, Bonnefond A, Andersson-Assarsson JC, Sudmant. PH, Dorajoo R, Al-Shafai MN, Bottolo L, Ozdemir E, So HC, Davies RW, Patrice A, Dent R, Mangino M, Hysi PG. Low Copy number of the salivary amylase gene predispose to obesity. Nat Gen. 2014;46(5):492-7. https://doi.org/10.1038/ng.2939
6. Hansen TV, Simonsen MK, Nielsen FC, Hundrup YA. Collection blood, saliva and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. Can of Epidem Bio and Preven. 2007;16(10):2072-6. https://doi.org/10.1158/1055-9965.EPI-07-0611
7. Peng JI, Jayapal SR, Lodish HF. Enucleation of cultured mouse fetal erythroblasts requires Rac G TPases and mDia 2. Nat Cel Biol 2008:10:314-32. https://doi.org/10.1038/ncb1693
8. Gaza-Nurun NS, Mohammad ZS. Mitochondrial DNA and methods for forensic identification. Jour of Foren Sci and Cri Inves. 2018;9(1):555755-9.
9. Tan VM, Ooi DS, Kapur J, Wu T, Chan, YH, Henry CJ, Lee, YS. The role of digesting factors in determining glycemic response in a multi ethnic Asian population. Euro Jour of Nutri. 2016;55(4):1573-81. https://doi.org/10.1007/s00394-015-0976-0
10. Tolmachov OE. Transgenic DNA modules with pre-programmed self-destruction: Universal molecular devices to escape 'genetic litter' in gene and cell therapy. Med Hypo. 2015;85(5):686-99. https://doi.org/10.1016/j.mehy.2015.08.012
11. Grady JP, Pickett SJ, Ng YS, Alston, CL, Blakely EL, Hardy SA, Feeney CL, Bright AA, Schaefer AM, Gorman GS, McNall RJ, Taylor RW, Turnbull DM, McFarland R. mtDNA heteroplasmy level and copy number indicate disease burden in mitochondrial disease. EMBO Mol Med. 2018;10(6):e8262. https://doi.org/10.15252/emmm.201708262
12. Amit B, Edward JR, Anshul K, Charles GD, Dan M. Initiation of mtDNA transcription is followed by pausing and diverges across human cell types and during evolution. Gen Res.2017;27(3):362-73. https://doi.org/10.1101/gr.209924.116
13. Michael JO, Keith AS, Daniel LK . Genetics, genomics and their relevance to pathology and therapy. Bes Prac & Res. Clin Rheuma. 2015;28(2):175-89. https://doi.org/10.1016/j.berh.2014.05.001

14. Fowler RF, Skinner DM. Eukaryotic DNA diverges at a long and complex pyrimidine: purine tract that can adopt altered conformation. The Journ of Biol Chem. 1986;261(19):8994-9001. https://doi.org/10.1016/S0021-9258(19)84479-4
15. Garrido-Ramos MA. Satellite DNA in Plants: More than Just Rubbish. Cyto & Gen Res. 2015;146:153-170. https://doi.org/10.1159/000437008
16. Lohe AR, Roberts PA. Evolution of satellite DNA sequences. In: Verma RS, editor. Drosophila in Heterochromatin: Molecular and Structural Aspects. Cambridge: Cambridge University Press. 1988. p.76-82.
17. Plohl M, Meštrović N, Mravinac B. Satellite DNA evolution. Repetitive DNA. 2012;7:126-152. https://doi.org/10.1159/000337122
18. Maria L, Carneiro V, Luciane S, Augusto L, Carla FM. Microsatellite markers: what they mean and why they are so useful. Gen & Mol Biol. 2016;39(3): 312-328. https://doi.org/10.1590/1678-4685-GMB-2016-0027
19. Gous M, Mohd YR, Mohd RI, Adam BP, Harun AR, Khan NI, Mohammad AL. A Review of Microsatellite Markers and Their Applications in Rice Breeding Programs to Improve Blast Disease Resistance. Intern Jour of Mol Sci. 2013;14:22499-528. https://doi.org/10.3390/ijms141122499
20. Gaza Nurun NS, Mohammad ZS. Mitochondrial DNA and methods for forensic identification. Jour of Fore Sci and Cri Inves. 2018;9(1): 555755-9.
21. Innis MA, Myambo KB, Gelfand DH, Brow MA. DNA sequencing with thermos aquaticus DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. PRONAS. 1988;85(24):9436-40. https://doi.org/10.1073/pnas.85.24.9436
22. Avinash M, Anurag KS, Gaur RK. Molecular Markers: Tool for Genetic Analysis. Anim Biotech. 2014;1:289-305. https://doi.org/10.1016/B978-0-12-416002-6.00016-X
23. Virkler K, Lednev IK. Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Foren Sci Intern. 2009;188(1-3):1-17. https://doi.org/10.1016/j.forsciint.2009.02.013
24. Gill P. An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes. Intern Acad of Leg Med. 2001;114(4-5):204-10. https://doi.org/10.1007/s004149900117
25. Clayton TM, Whitaker JP, Sparkes R, Gill P. Analysis and interpretation of mixed forensic stains using DNA STR profiling. Foren Sci Intern. 1998;91:55-70. https://doi.org/10.1016/S0379-0738(97)00175-8
26. Minari JB., Adebiyi B, Feyikemi D, Adetuyi SO, Umoffia EV. Investigation of DNA from traces of saliva extracted from cigarette butts of smokers in Lagos exposed to

different experimental conditions using STR markers. Journ of Sci Res & Dev. 2021;20(1):15-24.

27. Bowers CM. Problem-based analysis of bitemark misidentifications: the role of DNA. Foren Sci Intern. 2006;159(1):104-9. https://doi.org/10.1016/j.forsciint.2006.02.032
28. Reitsma MB, Kendrick PJ, Ababneh E, Abbafati C, Abbasi-Kangevari M, Abdoli A, Abedi A, Abhilash ES, Abila DB, Aboyans V, Abu-Rmeileh NM, Ahmad S, Ahmadi K.. Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and attributable disease burden in 204 countries and territories, 1990-2019: a systematic analysis from the Global Burden of Disease Study 2019. The Lancet. 2021;397:2337-60. https://doi.org/10.1016/S0140-6736(21)01169-7
29. American Cancer Society, Vital Strategies. The Tobacco Atlas: [Nigeria Fact Sheet]. https://files.tobaccoatlas.org. Accessed October, 10 2021.
30. National Bureau of Statistics, Federal Ministry of Health, World Health Organization Regional Office for Africa, CDC Foundation, Department of Health and Human Services USA, Centers for Disease Control and Prevention . GATS Nigeria. Global Adult Tobacco Survey: Country Report 2012. http://www.nigerianstat.gov.ng. Accessed September 28, 2021.
31. African Tobacco Control Alliance. The sale of single sticks of cigarettes in Africa. https://atca-africa.org/en/the. Accessed August 20, 2021.
32. Ijeoma UI, Chika NO, Deborah O, Benjamin SCU, Scott M, Emmanuel NA, Sijiu W, Echezona EE. Predictors of current tobacco smoking by adolescents in Nigeria: Interaction between school location and socioeconomic status. Toba Indu Dise. 2020;18:13-9. https://doi.org/10.18332/tid/117959
33. Aguirre A, Testa-Weintraub, LA, Banderas, JA, Haraszthy, GG, Reddy, MS, Levine MJ. Sialochemistry: A diagnostic tool? Critic Rev in Oral Biol and Med. 1993;4:343-50. https://doi.org/10.1177/10454411930040031201
34. Bianchi L, Liò P. Forensic DNA and bioinformatics. Bri Bioinforma. 2007;8(2):117-28. https://doi.org/10.1093/bib/bbm006
35. Van SK, De-Ceuleneer M, Dhaenens, M, Van. HD, Deforce D. Mass spectrometry-based proteomics as a tool to identify biological matrices in forensic science. Intern journ of leg med. 2013;127(2):287-98. https://doi.org/10.1007/s00414-012-0747-x
36. Abraham JE, Maranian MJ, Spiteri I, Russell R, Ingle S, Luccarini C, Earl HM., Pharoah PD, Dunning AM, Caldas C. Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. BioMed Cent Med Gen. 2012;5:19-24. https://doi.org/10.1186/1755-8794-5-19

37. Shailja C. Saliva as a forensic tool. Journ of Foren Dent Sci. 2019;11(1):1-4. https://doi.org/10.4103/jfo.jfds_69_18
38. Susmita S, Sanjeev, K.. Saliva in forensic odontology: A comprehensive update. Journ of ora and maxillof patho. 2015;19(2):263-65. https://doi.org/10.4103/0973-029X.164549
39. Parson W, Gusmão L, Hares DR, Irwin JA, Mayr WR, Morling N, Pokorak E, Prinz M, Salas A, Schneider PM, Parsons TJ. DNA Commission of the International Society for Forensic Genetics: revised and extended guidelines for mitochondrial DNA typing. Foren Sci Intern Gen. 2014;13:134-42. https://doi.org/10.1016/j.fsigen.2014.07.010
40. Bonanomi G, Incerti G, Cesarano G, Gaglione SA, Lanzotti V. Cigarette Butt Decomposition and Associated Chemical Changes Assessed by 13C CPMAS NMR. PlosOne. 2015;10(1):e0117393. https://doi.org/10.1371/journal.pone.0117393
41. Haske-Cornelius O, Pellis A, Tegl G, Wurz S, Saake B, Ludwig R, Sebastian A, Gibson S, Nyanhongo GS, Georg M, Guebitz GM. Enzymatic Systems for Cellulose Acetate Degradation. Catalyst. 2017;7(1):287-95. https://doi.org/10.3390/catal7100287
42. Hedman J, Dalin E, Rasmusson B, Ansell R. Evaluation of amylase testing as a tool for saliva screening of crime scene trace swabs. Foren Sci Intern Gen. 2011;5:194-8. https://doi.org/10.1016/j.fsigen.2010.03.003
43. Casey L, Engen S, Frank, G. Quantitative Analysis of the DNA Distribution on Cigarette Butt Filter Paper. Journ of Foren Sci. 2013;58(2):1-4. https://doi.org/10.1111/1556-4029.12091