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Forensic Examination Of Bite Marks Present On Different Surfaces

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ABSTRACT

As technology continues to evolve, more precise and prompt methods of performing investigations are required. The importance of bite mark analysis as a forensic tool for legal investigation thus continues to grow.

Bite mark evidence is commonly found in various crimes. A thorough knowledge and analysis of bite marks can help not only in the acquittal of the innocent but also provide conclusive evidence for conviction of criminals. Most often the bite mark injuries are present on skin. Sometimes, these marks may also be present on inanimate objects including various food stuffs.

Diverse characteristics (palatal measurements) of a human bite mark, including height of the arch, incisor- incisor and inter-canine distances were metered with the help of an ABFO scale. The existence of a statistically significant sexual dimorphism in mandibular ($p<0.05$) and maxillary ($p<0.05$) inter-canine distances measured from bite marks on different substrata (except in case of cheese where $p=0.12$) was concluded. In contrast, sexual dimorphism in mandibular ($0.05>p<0.14$) and maxillary ($0.05>p<0.35$) incisor-incisor distance as well as the height of arch ($0.05>p<0.9$) was found to be insignificant. It is also found that some amount of saliva (containing a large number of buccal epithelial cells) is left behind in the bite marks; which can be used to determine the sex of the perpetrator by examining the presence of Y or Barr body. So, in the present study, encouraging progress has been made towards determination of sex from bite marks for individualization purpose.

INTRODUCTION

Bite marks are regularly found in a number of crime cases like sexual assault, quarrels, homicide and sometimes in burglary. Biting is used by human beings both for offence and self defense. These marks are also found on food, flesh or in variety of materials like cigar, cigarette buds, musical instruments, etc.

Bite marks are as individual specific as fingerprints; hence they are sometimes called *'Dental Fingerprint* '(1). Several characteristics such as missing teeth, gaps between teeth, malposed teeth or rotated teeth, tooth width, tooth thickness, jaw width etc. can be used to establish the identity of an individual. During the process of bite mark analysis the class and individual characteristics of the suspected biter's dentition is compared with the bite mark from the scene of crime. (2).

Most often the bite mark injuries are present on skin. But skin is a poor registering material due to its high visco-elasticity, variability in terms of anatomical location, underlying musculature or fat, curvature, and looseness or adherence to underlying tissues. A number of inanimate objects have been used for accurate registration of bite marks (3).

Various Scientists have made various efforts to identify the Tooth and Arch mark in bite marks by using different techniques such as Plaster of Paris, silicone or synthetic rubber elastomer and metal spraying to produce 3-dimensional model of biters teeth (4); X-Y digitizer and a computer for determining size and relative positions of anterior teeth (5), Microbiologic and histologic/ histochemical techniques were also tried to further delineate the nature of the bite marks and to aid in the identification(6). Scientists have made varied attempts to maintain the dimensional integrity of bite marks; Formol acetic alcohol (FAA) mixture was used as preservative of bitten fruits (apple) (7). ABFO (American Board of Forensic Odontology) No.2 was developed by Hyzer and Krauss (1988) for accurate scaling and facilitates gridding of photographs to correct distortional errors (8). Accuracy of bite mark measurement from skin is very important to record the uniqueness of human dentition (9). Tooth size standards based on odontometric investigations can be used in determination of age and sex (10). Mandibular canine index and maxillary central incisors measurements based on morphometric analysis have been used for sexual dimorphism studies in North Indian population (11, 12). The uniqueness of the anterior dentition has been studied by analyzing the size of incisor, canine occlusal surface and shape variation using geometric morphometric techniques based on landmark and semi-landmark data (13).

The measurement of bite marks is normally performed with ABFO scale. The unique L-shaped configuration of ABFO scale (Photograph-1) ensures comparatively accurate scaling in both vertical and horizontal directions and facilitates gridding of photographs to correct for distortional errors created by oblique camera angles (14). Keeping in mind the advantages, all the measurements of bite marks in the present study were made with the ABFO Scale (8, 15).

Bite marks have traces of saliva containing a number of buccal epithelial cells. However, stains of dried saliva left on bite marks are invisible, which adds to the difficulty of recognizing and collecting from the surface. The DNA extraction from saliva traces collected from skin is more difficult than from similar stains on clothing, paper or other inanimate objects, since the substrate on which the saliva is deposited (skin) cannot be used directly for extraction procedures.

An efficient collection method is required to collect saliva/ saliva stains from bite marks present on human skin and other substrate, as quantity present is often minimal. Contamination by DNA from the skin is also a potential problem. The double swab technique which employs a wet cotton swab

followed by a dry cotton swab has been shown to increase the amount of salivary DNA recovered from skin when compared to other methods (16). The standard procedures used for extraction of DNA, Phenol-chloroform extraction, takes a relatively long period of time, is expensive and involves the use of toxic and hazardous chemical reagents but it produces consistent results (17) so some modifications are made in the extraction process.

Bite marks also help in determining the sex of an individual by noting the shape of the arch; in males it is narrow U-shaped whereas in females it is wider and more elliptical. Bite marks contain traces of saliva, containing buccal epithelial cells which on staining can help in determination of sex and individualization by studying the presence of Barr body (18), Y-body (19, 20) and DNA (21).

The identification of Barr body, Y-body and extraction of DNA can be done from very small amount of salivary cells present on and around the bite marks. So in the present study, an attempt has been made to extract the DNA and visualize Barr body and Y-body, besides making morphometric measurements with the help of ABFO scale on different food stuffs with the help of different collection procedures.

Materials and Methods

In the present study, bite marks samples were collected on different food stuffs from 30 subjects, out of which 24 individuals were with *normal arch relationship* and 6 individuals were with *mandibular prognathism*. Age, sex, and caste etc. of all the subjects were recorded in a separate register and sample number was allotted.

Normal Arch Relationship

According to Angle Classification it is defined as Mesiobuccal cusp of maxillary molar falling into the midbuccal groove of mandibular molar. In these cases arch relationship is normal with mandible within the confines of maxillae.

Mandibular prognathism (Projection of lower jaw**)**

It is a genetic disorder where the lower jaw outgrows the upper, resulting in an extended chin

Selection of samples

Out of 30 samples, 12 males and 12 females samples having *normal arch relationship* and remaining 3 males and 3 females' samples with *mandibular prognathism* were collected on the following different types of food stuffs-:

- Apple
- Guava
- Cheese
- Chocolate

The following two techniques were applied to collect saliva samples from bite marks separately:

1) Saline Washing Technique

In this technique, the bite mark area was washed with normal saline and the resulting saliva solution was collected in Petri dish. Following high speed centrifugation, the supernatant was discarded and the residue was observed under a microscope.

2) Double Swab Technique

In this technique, the first swab was wetted by immersing its cotton tip in saline solution and then rolled over the surface of bite mark thoroughly using moderate pressure in circular motion. The swab was left for total air drying. The second swab (without moistening) was used in a way identical to the first one and air dried.

Both the swabs were dropped in saline taken in a test tube, allowing the salivary epithelial cells to enter the saline solution and mix thoroughly. This solution (containing saliva cells) was centrifuged at high speed and kept in refrigerator until used (16).

The bite marks were photographed by putting ABFO scale for the precise measurement of bite marks.

MORPHOMETRIC MEASUREMENTS

The following measurements of bite marks were taken with the help of ABFO scale.

1) ***Maximum Height of an Arch*** **(length of bite)-*:*** It is a straight distance between highest point of upper jaw and lowest point of lower jaw on bite mark.

2) ***Maximum Breadth of an Arch i.e. Jaw width*** **(canine to canine distance)-:** It is a distance between two canines of same jaw (Canine is chosen as reference because these are the most sideward placed points most commonly present in bite marks).

3) Incisor to Incisor distance-: Distance between distal line angles of two incisors (from left side of left incisor to right side of right incisor); done for both jaws.

4) Noted the individual characteristics such as Missing tooth, Gap, Malposed, Rotated teeth, etc if any.

The following statistical calculation (as mentioned in Table-1-18) was performed:

Mean, Standard Deviation, Variance, t-test was calculated for each variable for each subject (Table-1-18). The p-value was calculated by using the following software (http://www.danielsoper.com/statcalc/calc08.aspx)

SEX DETERMINATION

As mentioned above, the saliva sample of 30 subjects (15 males and 15 females) were collected from bite marks taken on different food stuffs (apple, guava, chocolate, cheese) by different collection procedures (Double Swab technique and Saline Washing). Sex was determined by examining Barr body and Y-Body Fluorescence as follows:

Examination of Barr body: To determine the Barr body (which is mostly present in females) from the salivary epithelial cells, the orcein dye is required. The orcein solution was prepared as follow:

Preparation of Aceto-orcein Stain

In a conical flask, 100 cc of Acetic acid and 1.3gm of Orcein dye powder were taken and dissolved in Acetic acid. The contents were shaken thoroughly and the mixture was refluxed for 2 hours. The solution was centrifuged after refluxing and then filtered. The final solution was labeled as Aceto-orcein stain. It was stored in a dark bottle and kept in a dark and cool place.

Y-Body Fluorescence

To determine the **Y-Body Fluorescence,** which is mostly present in males and is a conclusive test for determination of sex from the salivary epithelial cells, the Quinacrine dye is required (5 mg/ml solution of quinacrine dihydrochloride dissolved in water).

Preparation of Slides

After the collection of saliva sample the supernatant is rejected and the residual drop is placed on the sterilized microscopic slide. The cells were spread with another microscopic slide at an angle of 40^{o}-45^{o} to make a smear on the slide and allowed to fix. In this way all the saliva samples were prepared for staining as follows:

Staining the Microscopic Glass Slide

A drop or two of Aceto-orcein stain was placed on each slide with the help of dropper. It was spread and left as such for a minute or two. The slides were heated over a spirit lamp for 10-20 seconds at about 60^{o} C. Again, one or two drops of Aceto-orcein stain were poured, the slides were covered with cover slip and thumb was used to squeeze out the excess of stain in the folds of filter paper. The slides were examined under low (10X x 10X) and high power (10X x 40X) of microscope, many cells was seen on the slide and percentage of nuclei containing the dark brown Barr body on the nuclear wall was calculated (22, 23).

Similar procedure was also adopted for determination of Y-Body Fluorescence, but in this case the microscopic slide was examined under Fluorescence Microscope. The pin tip sulphur yellow fluorescence of Y-Body was clearly visible and percentage of the cell containing Y-Body was also calculated.

DNA EXTRACTION

Saliva samples were extracted from the bite marks taken from 10 subjects with the help of *Saline washing collection* technique, and transferred into sterile vials (Eppendorf tubes) which were stored in refrigerator at 4^{0} C until use.

Solution Used and their Composition

1). 1X PBS (Phosphate Saline Buffer) -:

Na_2HPO4 =5.75g

NaH_2PO4 =1.482g

NaCl =9.0g

Total volume=1L

2). CLB (Cell Lysis Buffer)-:

Tris HCl =40mM

$MgCl_2$ =20mM

Triton X =4.0%

Autoclaved and add

Sucrose =1.28M

3). NLB (Nucleic Acid Lysis Buffer) (400ml) -: (pH-8.0)

Tris HCl =10mM

NaCl =400mM

EDTA =2.0mM

4).Tris EDTA Buffer (200ml) -:

Tris =10mM

EDTA =1mM

5). 10% SDS (Sodium Dodecyl Sulphate)

6). Proteinase K =75 µg/ml

7). 5M NaCl (Sodium chloride)

8). Chilled ethanol.

Procedure of DNA Extraction

200µl of saliva sample collected by *Saline Washing* of the bite mark area was taken into the Eppendorf tube and added to it 1ml of PBS and 800ul of CLB. Then it was incubated at 4^0C for 20 minutes followed by centrifugation at 5000 rpm for 20 minutes. The supernatant was discarded and pellet saved. To the pellet, Proteinase-k, 800 µl of SDS and 1ml of NLB was added. It was incubated at 65.5^0 C for 3.5 hours. Then 1ml of freshly prepared NaCl was added and centrifuged again at 5000 rpm for 20 minutes. The supernatant was taken and double its volume of chilled ethanol was added to it before spooling out the DNA.

Determination of Concentration of DNA by U.V Spectrophotometry

The concentration/ purity of isolated DNA were determined by using U.V Spectrophotometer at 260 and 280 nm. The Nitrogenous bases in double stranded DNA exhibited strong absorption maximum at a wavelength of 260 nm. As Optical Density $(O.D)_{260}$ corresponds to DNA content and $(O.D)_{280}$ corresponds to protein content. The ratio between readings 260 and 280 ($O.D_{260/280}$) provided an estimated purity of DNA.

Agarose Gel Electrophoresis

Gel electrophoresis was employed so as to determine the quality of DNA extracted.

TBE Buffer (Tris Boric Acid and EDTA)

Tris Boric Acid =0.9M

Boric Acid =0.9M

EDTA =0.02M

0.8% solution of agarose was made in TBE Buffer. This solution was prepared in conical flask. The neck of flask was plugged loosely and the slurry was heated at 60-70^0 C till agarose was dissolved completely. To this solution Ethidium Bromide was added to a final concentration of 0.5 ug/ml and was mixed well. It was made sure that there was no air bubble in gel solution.

Electrophoretic plate was leveled by using a leveler. The above prepared gel solution was poured into the gel casting electrophoretic plate in a single slot without a break. Immediately after pouring the gel, toothed comb was placed in the plate. This plate was kept undisturbed for one hour to solidify the gel. Once the gel got solidified, comb was removed from it with great care.

The cool gel plate was then placed in electrophoresis chamber. The chamber was filled with enough electrophoresis buffer i.e. TBE Buffer to submerge the gel to depth of about 1mm.The DNA sample was mixed with gel loading dye i.e. Bromophenol blue. After thorough mixing, the mixture was loaded into wells of submerged gel and likewise all samples were mixed and loaded.

The gel was allowed to run at 50V for 1 hour. The power supply was then turned off. The plate was taken out and the gel was visualized under U.V transilluminator and photographed.

RESULTS and DISCUSSION

The samples of the bite marks on the different food stuffs were taken and analyzed for the following parameters:

MORPHOMETRIC MEASUREMENTS

The results of morphometric measurements taken by ABFO scale (photograph-1) include maximum height, maximum breadth of Arch, Distance between distal line angle of two incisors, and individual characteristics (if any) on different food stuffs such as Apple, Guava, Chocolate and Cheese are given in Table 1 - 18. The canine to canine distance in the male upper jaw varies from 30 to 38mm (table-19) while in females, this distance varies from 29 to 36mm (table-20); Whereas the distance between the distal line angle of two incisors varies from 10 to 17mm (table-19) and 10 to 18mm (table-20) in upper jaw of male and female respectively. This indicates that there is existence of a statistically significant sexual dimorphism in the morphometry of the mandibular and maxillary inter canine distances as compared to incisor to incisor distance and is found to be insignificant for the parameter Maximum Height of an Arch (length of bite). . These finding are consistent with that of work done by other workers while studying with dental casts (24, 25, and 26).

Besides 12 samples (of each sex) of normal arch dentition (photograph-2), 3 samples of male and female each with mandibular prognathism (photograph-3) were also analyzed. In contrast to our expectation the average distance between canine to canine in both male and female was less in lower jaw as compared to upper jaw (table 21 and 22); Whereas the distance between distal line angle of incisor to incisor also exhibited the same pattern (table 21 and 22).

The Shape of Arch has a characteristic pattern which can be used to distinguish between male and female and is evident from the photographs (4-11) taken on different food stuffs and even some of the individual characteristics are clearly visible in photographs (12, 13).

SEX DETERMINATION

It is evident from photograph (14) that the Barr body is present in most of the females which is in concordance with the previous work (22). The presence of Barr body is expected in females because they contain 2X-Chromosomes, but the Barr body may also be present in a few male samples, which have genotype of XXY. The percentage of cells having Barr body varies from 55-65%. This means that theoretically, presence or absence of Barr bodies can be used to determine the sex only up to an extent because of the following reasons:

- In cases of *Turner Syndrome* 'XO' in females, Barr bodies are absent.
- *Klienfilter males* possessing more than one X- Chromosome would show presence of Barr body.

However, the percentage of the two populations is too scant to be of much statistical significance. The presence of Y-Body corroborates the male origin of a sample beyond any doubt; hence this can be used as confirmatory test to determine sex from saliva recovered from or around the bite marks.

DNA EXTRACTION

In the present study, the feasibility of DNA extraction from saliva samples (collected from bite marks and surrounding area) using different techniques such as double swab technique and saline washing has been studied. Of these, the saline washing was preferred, particularly when DNA is required to be extracted from bite marks present on various food stuffs.

Further the modified extraction method was performed in which NaCl is used to "salt out" proteins from within the samples. It gives comparatively better results as compared to other extraction methods by omitting the hazardous use of Phenol-chloroform method and gives better yield as compared to chelex method of extraction (27, 28, 29)

The absorbance of DNA sample at 260nm & 280nm is shown in table-23. The ratio between the absorbance reading at 260 nm & 280 nm provides an estimate of the purity of nucleic acid. The absorbance ratio listed in table- 23 indicates that the samples are somewhat contaminated with proteins. The $A_{260/280}$ value of samples is lower. Since all samples were treated with Proteinase-K for 3 hours and 30 minutes, absorbance ratio indicates that it still requires further treatment to remove the left over proteins. However it is evident from result that genomic DNA could be extracted from saliva collected from bite marks and surrounded area, with Saline Washing Technique which gives equivalent results as in double swab technique. The results of DNA extraction and determination with the modified technique are similar to those reported in previous studies (21).

The result of agarose gel electrophoresis clearly shows that DNA could be extracted from saliva collected from bite marks. The bands could be clearly visible in photograph 15.

The great variations exist in the length of the bite marks every time in the same individual. In male samples, it varies from 41 to 50mm (table - 19) in case of guava and 42 to 51mm in case of apple. Almost similar length was observed in female samples (table - 20). Whereas it is consistent in case of inter-canine distances and comparatively lesser variation in incisor-incisor distances when taken on different food stuffs of same individual.

Besides 12 samples (of each sex) of *normal arch dentition*, samples of 3 males and 3 females with *mandibular prognathism* were also analyzed. In contrast to our expectation, the average canine to

canine distance both in males and females, was less in the lower jaw than the upper jaw (table 21 and 22); The distance between the distal line of incisors also exhibited the same pattern (table 21 and 22). The metrics of arch width (inter-canine distance) both in the maxillary and mandibular regions are greater in males than in females. The sexual dimorphism in morphometry for mandibular as well as maxillary intercanine distances for different substrata is highly significant ($p<0.05$), except in case of cheese where $p=0.12$. On the other hand, incisor-incisor distance had a low sexual dimorphism in both mandibular ($0.05>p<0.14$) and maxillary $p<0.35$ regions. The height of arch from bite marks of males and females on different food stuffs do not give a statistically significant result ($p<0.9$). Hence it cannot be a good criterion for sex determination.

It is also seen that the shape of arch has a characteristic pattern which can be used to distinguish between males and females. It is evident from the photographs (4-11) taken on different food stuffs.

Along with morphometric measurements, the saliva samples collected by saline washing technique were analyzed for presence of Barr bodies (in case when saliva sample is from a female) and a successful attempt to extract DNA from the traces of saliva sample was done.

BLIND SAMPLES TESTED FOR IDENTIFICATION BY MORPHOMETRIC ANALYSIS

Bite mark analysis is a vital area within the highly specialized field of Forensic science and constitutes the commonest form of dental evidence presented in criminal court.

In the present study a blind trial was organized to identify the person from bite marks with the help of different techniques using life size photographs; morphometric measurements; transparent overlays; from study dental cast models in different materials such as fruits, chocolate and cheese. The findings are consistent with that of work done by other authors (5, 30).

Direct comparison -: In this method, morphomertic analysis of 15 unknown photographs on fruits; chocolate and cheese with 15 dental casts of the suspects was performed and concordant point were matched directly. Hence, the model was moved on the photograph for comparison.

Indirect comparison-: In this method, transparent overlay was placed directly over the bite marks on photographs and the match was noted.

Overall analysis-: To identify the characteristics of bite marks by morphometric analysis and comparison of dental cast or transparency with photograph, then only expert comments could be given about the match was positive or else.

Match positive

There were no doubts about the bite marks when compared with dental casts it was termed as positive match.

Match Non–contributory

In case the dental cast did not match at all with photograph of bite marks or there was some degree of doubt due to technical fault in making of cast or photograph, then the comment of expert was reserved as match non–contributory rather than match negative.

Table 24. Bite Marks Matches. Results of blind samples tried for identification

Sr. No	Bite marks on	No. of cases	Direct	Indirect	Positive	Non-contributory
1.	**Fruits**	15	14	14	14	7%
2.	**Chocolate**	15	13	13	13	13%
3.	**Cheese**	15	12	12	12	20%

Bite marks on the fruits, the match was positive in 95 per cent while it was positive in 87 percent in chocolate and 81 per cent in cheese. In comparing the direct and indirect method of match, it was found that MORPHOMETRIC MESUREMENTS can be good criteria for matching bite marks as evident from table-24.

SUMMARY AND CONCLUSION

From the present investigation, it is evident that morphometric analysis can play an important role in the identification of suspects and help in distinguishing between males and females. Similarly, Barr bodies also have a role in sex identification as usually present in normal females. But conclusive evidence regarding sex identification can be obtained from Y-chromosome.

This study shows that even from such a small quantity of saliva collected from bite marks, extraction of DNA is feasible with inorganic extraction method by using NaCl. But, further studies are needed to reach to final conclusion.

TABLES

Table 1. Measurement showing length of bite in bite marks of Male and female taken on different food stuffs on Apple (in mm)

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Jaw length	Female	44.83	3.325	7.418	11.05563	12	0.125	21.9	0.9	not significant
	Male	44.67	3.2	7.165	10.24	12				

Table-2 Measurement showing length of bite in bite marks of Male and female taken on different food stuffs(on gauva). (in mm)

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Jaw length	Female	44.83	3.325	7.418	11.05563	12	0.125	21.9	0.9	not significant

Table-3

Comparison of measurement (in mm) for parameter canine to canine distance between male and female in upper jaw on Apple

Parameter	Sex	Average	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Maxillary intercanine distance	Female	32.167	1.9924	6.19402	3.9697	12	1.697	21.96601	0.052	significant
	Male	33.333	2.0724	6.1724	0	12				

Table-4

Comparison of measurement (in mm)for parameter canine to canine distance between male and female in upper jaw on Gauva

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Maxillary intercanine distance	**Female**	32.117	1.9544	6.085328	3.8197	12	1.9063	21.92768	0.0348	**Highly significant**
	Male	33.683	2.0701	6.145653	4.28515	12				

Table-5

Comparison of measurement (in mm)for parameter canine to canine distance between male and female in upper jaw on Chocolate

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance

Parameter	Sex	Average	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Maxillary intercanine distance	**Female**	32.133	1.9727	6.139084	3.89152	12	1.8124	21.9778	0.04181	**Highly significant**
	Male	33.617	2.0364	6.057742	4.14697	12				

Table-6

Comparison of measurement (in mm) for parameter canine to canine distance between male and female in upper jaw on Cheese

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Maxillary intercanine distance	**Female**	32.7	2.0675	6.322622	4.27455	12	1.2034	21.8105	0.12081	**Highly significant**
	Male	33.767	2.2701	6.722889	5.15333	12				

Table-7

Comparison of measurement (in mm)for parameter canine to canine distance between male and female in lower jaw on Apple

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Mandibular intercanine distance	Female	28.583	1.4434	5.049711	2.08333	12	3.3489	21.77006	0.00133	**Highly significant**
	Male	30.667	1.6002	5.218009	2.56061	12				

Table-8

Comparison of measurement (in mm)for parameter canine to canine distance between male and female in lower jaw on Guava

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Mandibular intercanine distance	**Female**	28.6	1.4245	4.980635	2.02909	12	3.7709	21.60409	0.00053	**Highly significant**
	Male	30.958	1.6323	5.272636	2.66447	12				

Table-9

Parameter	Sex	Average	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance

Comparison of measurement (in mm) for parameter canine to canine distance between male and female in lower jaw on Chocolate

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Mandibular intercanine distance	**Female**	28.6	1.4245	4.980635	2.02909	12	3.6414	21.80649	0.00072	**Highly significant**
	Male	30.825	1.5656	5.079029	2.45114	12				

Table-10

Comparison of measurement (in mm)for parameter canine to canine distance between male and female in lower jaw on Cheese

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Mandibular intercanine distance	**Female**	29.125	1.5393	5.285009	2.36932	12	2.8306	21.92462	0.00487	**Highly significant**
	Male	30.958	1.6323	5.272636	2.66447	12				

Table-11

Comparison of measurement (in mm) for parameter incisor to incisor distance between male and female in upper jaw on Apple.

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No. of samples	t test	Degree of Freedom	p' value	Significance
Maxillary I-I distance	Female	13.25	2.378884	17.95384	5.65909	12	0.504	21.28338	0.3	
	Male	12.70833	2.864027	22.53661	8.20265	12				

Table-12

Comparison of measurement (in mm) for parameter incisor to incisor distance between male and female in upper jaw on Gauva

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Maxillary I-I distance	Female	13.25	2.378884	17.95384	5.65909	12	0.534	21.2658	0.29928	
	Male	12.675	2.870896	22.65007	8.24205	12				

Table-13

Comparison of measurement (in mm)for parameter incisor to incisor distance between male and female in upper jaw on Chocolate

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Maxillary I-I distance	Female	13.30833	2.407359	18.08911	5.79538	12	0.57	21.35574	0.28713	
	Male	12.69167	2.869101	22.60618	8.23174	12				

Table-14

Comparison of measurement (in mm)for parameter incisor to incisor distance between male and female in upper jaw on Cheese

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Maxillary I-I distance	Female	13.225	2.364174	17.87655	5.58932	12	0.465	21.18597	0.32343	
	Male	12.725	2.883535	22.66039	8.31477	12				

Table-15

Comparison of measurement (in mm)for parameter incisor to incisor distance between male and female in lower jaw on Apple

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Mandibular I-I distance	**Female**	10.41667	0.668558	6.418156	0.44697	12	1.188	20.68652	0.1237	
	Male	10.04167	0.864931	8.613423	0.74811	12				

Table-16

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance

Comparison of measurement (in mm) for parameter incisor to incisor distance between male and female in lower jaw on Guava

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Mandibular I-I distance	Female	10.41667	0.668558	6.418156	0.44697	12	1.162	20.68606	0.12888	
	Male	10.05	0.864975	8.606717	0.74818	12				

Table-17

Comparison of measurement (in mm) for parameter incisor to incisor distance between male and female in lower jaw on Chocolate.

Parameter	Sex	12	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Mandibular I-I distance	Female	10.41667	0.668558	6.418156	0.44697	12	1.232	21.18857	0.11545	
	Male	10.04167	0.81515	8.117677	0.66447	12				

Table-18

Comparison of measurement (in mm)for parameter incisor to incisor distance between male and female in lower jaw on Cheese

Parameter	Sex	Average Mean	S.D	Coeff of variation	Variance	No of samples	t test	Degree of Freedom	p' value	Significance
Mandibular I-I distance	Female	10.41667	0.668558	6.418156	0.44697	12	1.112	21.64996	0.1389	
	Male	10.09167	0.759735	7.528338	0.5772	12				

Table 19. Showing measurement (in mm) between canine to canine in males on different food stuffs

Samples	Length of Bite	Range of the			
		Canine to canine		Incisor to incisor	
		Upper Jaw	Lower Jaw	Upper Jaw	Lower Jaw
Chocolate	-	30-38	29-34.7	10-20	9-11
Guava	41-50	30-38	29.34.5	10-20	8-11
Cheese	-	30-39	29-35	10-20	8.4-11
Apple	42-51	30-38	29-34.5	10-20	8-11

Table 20. Showing range of parameters of female of bite marks taken in to consideration during Morphometric measurements

Samples	Length of Bite	Range of the			
		Canine to canine		Incisor to incisor	
		Upper Jaw	Lower Jaw	Upper Jaw	Lower Jaw
Chocolate	-	29.5-34.5	27.2-31	10-18	10-12
Guava	41-49	30.2-36	27-31	10-18	10-12
Cheese	-	30-36.3	27-32	10-.15	10-12
Apple	40-49	29-36	27-31	10-18	10-12

Table 21. Results of palatal measurement of bite marks (in mm) (of males) with Mandibular Prognathism

Sample number	Upper jaw		Lower jaw		Individuality (if any)
	C-C	I-I	C-C	I-I	
BM_1	38	19	32	12	Gap between central incisors
BM_2	27	12	25	10	-
BM_3	34.5	12	30	11	-

Table 22. Results of palatal measurement of bite marks (in mm) of females with Mandibular Prognathism

Sample number	Upper jaw		Lower jaw		Individuality (if any)
	C-C	I-I	C-C	I-I	
BM_1	36	12	30	10	-
BM_2	35	11	30	10	-
BM_3	32	10	29	10	-

Table 23. Results of DNA from saliva collected from Bite marks studied with U.V Spectrophotometry

S.No	$^*\lambda_{260}$	$^{**}\lambda_{280}$	$\lambda_{260/280}$
1	0.013	0.007	1.857
2	0.005	0.004	1.256
3	0.017	0.013	1.307
4	0.007	0.008	0.875
5	0.013	0.009	1.444
6	0.004	0.004	0.947
7	0.011	0.011	0.991
8	0.007	0.007	0.985
9	0.003	0.005	0.519
10	0.012	0.009	1.333

$^*\lambda_{260}$ is absorbance at 260 and $^{**}\lambda_{280}$ is absorbance at 280

PHOTOGRAPHS



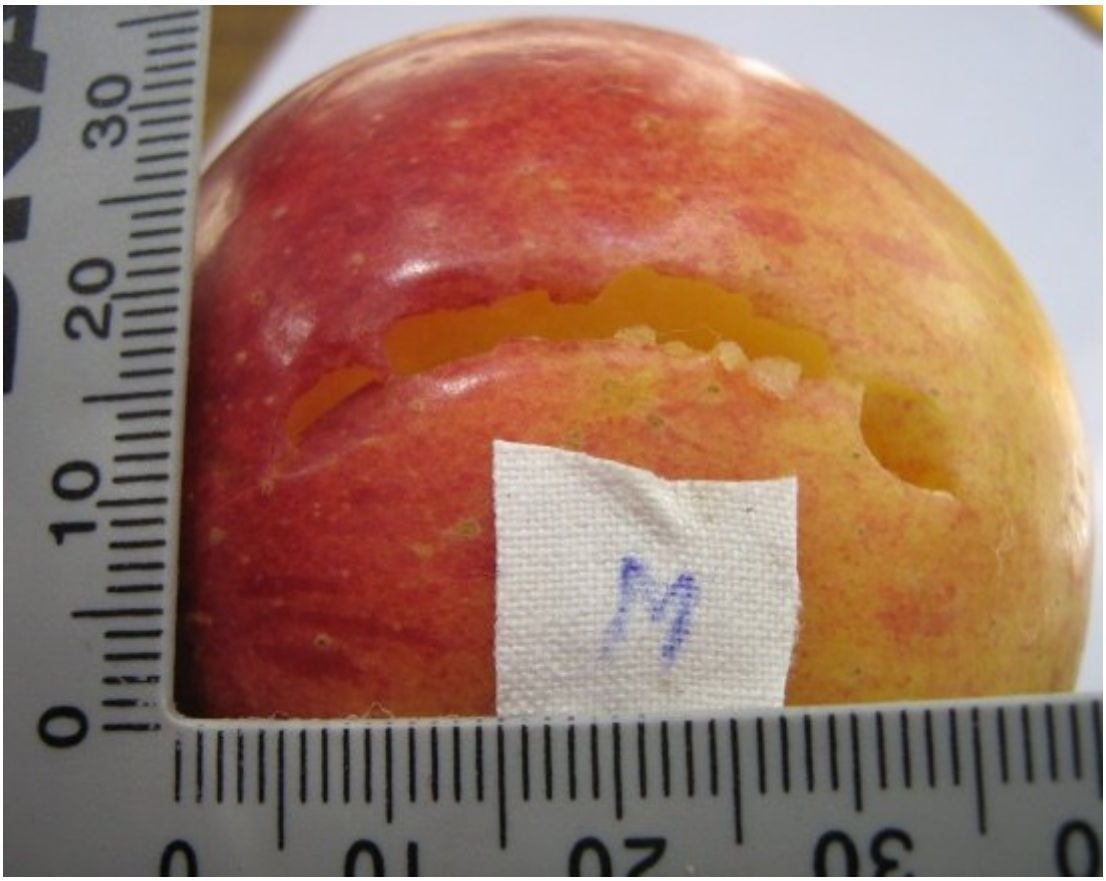
Photograph 1. Photographs Showing ABFO Scale Measuring Bite Marks on Apple



Photograph 2. Showing Normal Arch Dentition



Photograph 3. Showing Mandibular Prognathism



Photograph 4. Showing Bite Marks of male on Apple.



Photograph 5. Showing Bite Marks of Female on Apple



Photograph 6. Showing Bite Marks of Male on Guava and Malposed Teeth (Individual Characteristics)



Photograph 7. Showing Bite Marks of Female on Guava



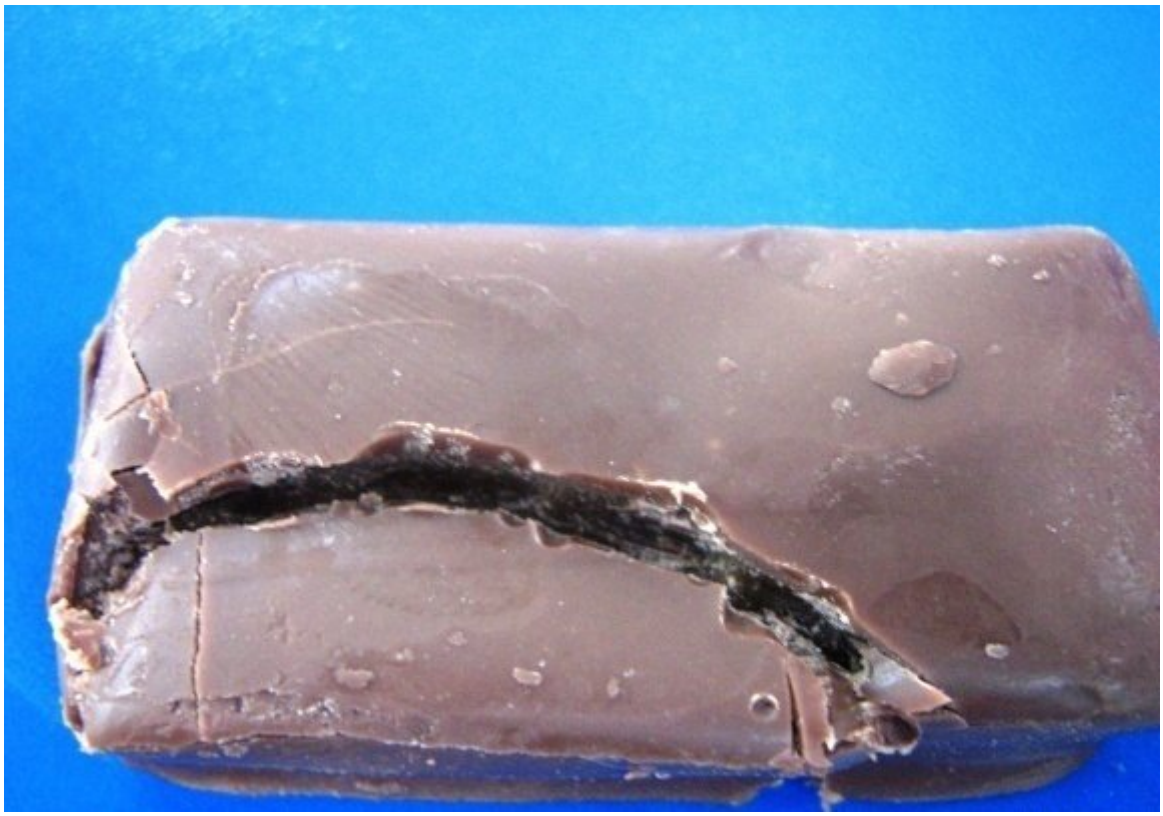
Photograph 8. Showing Bite Marks of Male on Cheese



Photograph 9. Showing Bite Marks of Female on Cheese



Photograph 10. Showing Bite Marks of Male on Chocolate



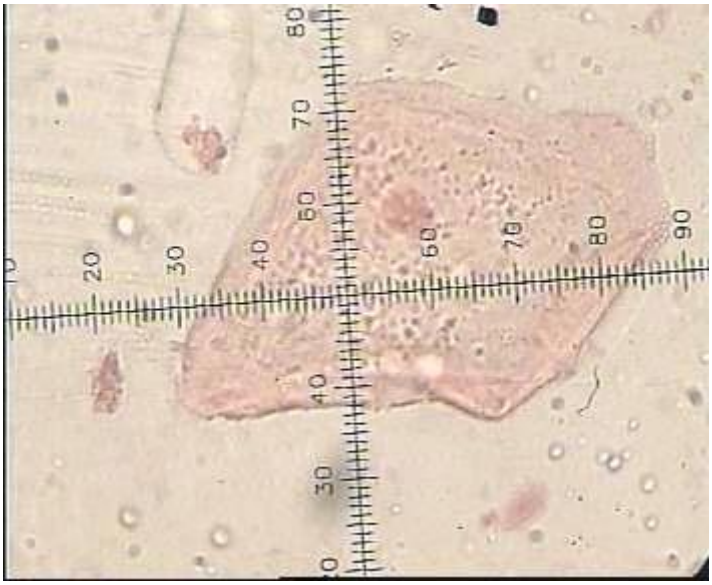
Photograph 11. Showing Bite Marks Of Female On Chocolate



Photograph 12. Showing Excessive Gap between Lateral Incisors **and Canine of Upper Jaw.**



Photograph 13. Bite Marks on Cheese Showing Excessive Gap between Lateral Incisor & Canine of Upper Jaw



Photograph 14. Showing the Presence of Female SalivaryEpithelial Cells Showing Presence of Barr body



Photograph 15. Showing result of Agarose gel electrophoresis

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