Introduction

Bite inflicted wounds are one of the most frequent forms of human trauma; humans have used their teeth as both tools and weapons since the dawn of time. Bite marks are usually seen in cases involving sexual assault, murder and child abuse, the assessment of which could be a major factor leading to conviction of the accused. Many violent assaults involve the presence of more than one bite, making some bites difficult to identify. Bite marks are accepted as being unique to each person since the characteristics of bite mark may be affected by the type, number, and peculiarities of the teeth, dynamics of occlusion, muscle function, individual tooth movement and temporomandibular joint dysfunction.[1]

Many techniques to analyze bite mark patterns have been used in the past. They involve the use of “overlay.” The tooth exemplar, independent of the method used to produce it when biting surface data are transferred to a clear acetate sheet, is called an “overlay.” These are physically compared to the injury on skin or a patterned mark. “Hollow volume overlay” records the perimeter of biting surface of each tooth and leaves the inner aspect of the tooth image transparent.[2]
Based on the site and type of bite marks, overlays are generated using hand tracing, xerographic images, or through X-ray films. These life-sized overlays can be compared with the overlays from suspect’s teeth. The present study aimed to assess the most accurate bite mark overlay fabrication technique by direct comparisons between models of cases and bite marks with indirect comparisons in the form of conventional traced overlays of subjects. It also aimed to determine the relative accuracy of the technique and its feasibility in forensic science.

According to Iain A. Pretty, the severity of a bite mark is an important factor in the assessment of the forensic significance of the injury and whether or not it can be compared with a suspect. The American Board of Forensic Odontology (ABFO) has published guidelines that describe the evidence that should be collected from both victim and suspect, and represent a sound basis for such collection. All of the photographs should be taken with the camera at 90° to the injury and DNA swabbing of the injury site should be a double swab – the first moistened with distilled water and the second dry.

**Materials and Methods**

Thirty subjects (10 males and 20 females) with complete set of natural upper and lower anterior teeth were selected for this study. Subjects with orthodontic appliances, intraoral prosthesis, loss of anterior tooth structure, or developmental tooth anomalies were excluded from the study. The upper and lower alginate impressions were taken from 30 subjects. Die stone model was obtained from each impression; overlays were produced from the biting surfaces of six upper and six lower anterior teeth using the following methods: hand tracing from study casts, wax impression method, radiopaque wax impression method, and by xerographic-based method. Following this, dental characteristics of the biting edge and degree of rotation of the six upper and six lower anterior teeth were measured. Area of tooth biting surface was included to evaluate differences in the relative length and breadth of recorded individual teeth and the width of the outline produced by each overlay method.

Overlay was produced by tracing the anterior teeth (maxillary and mandibular) on an acetate sheet, which was done using a fine-tipped felt pen by five techniques:
- Hand tracing technique: Hand tracing from study casts was done by keeping the acetate sheet on the biting surface of the upper and lower anterior teeth [Figure 1]
- Wax impression technique: A wax impression was taken on a sheet of modeling wax and the impressions were traced on an acetate sheet [Figure 2]
- Radiographic wax impression technique: Silver amalgam powder mixed with surgical spirit was added to the individual tooth impressions taken as above, A radiographic image was taken on an intraoral dental X-ray film. The film was processed; the bite marks showed as white teeth on a dark background. The radiographic image was then traced on a transparent sheet [Figure 3]
photocopy machine with incisal edges down. This was photocopied on an A4 sheet of paper. An acetate sheet was overlaid on the photocopy image of the casts and the outline of incisal edges was traced [Figure 4]

• 2D computer layout: The study casts were positioned on the 2D scanner plate with incisal edges contacting the plate and a color photograph was obtained. The saved image was imported into Photoshop (Adobe Photoshop 6 software) and was rotated to make the edge parallel to the x-axis of the computer. Selection of biting edges: The biting edge of teeth was highlighted by semi-automatic thresholding using magnetic lasso tool. Once the initial selections in all six teeth were done, the selection was smoothed and marked for comparison.[1] [Figure 5]

All the overlays were then subjected to measurement of area and angle of rotation of all 12 teeth. The scanned overlays were opened in Image J software, and the outlines of the tooth impressions were thresholded and a mask was created. The area, perimeter, as well as centroid coordinates for each tooth in each overlay were then obtained and tabulated. The centroid points were then marked for each tooth in Image J using the coordinates obtained. The centroids of the two central incisors were joined and a perpendicular was drawn at its midpoint. This was considered as the reference line to measure the angulation. Using the angle tool, the angle formed between the reference line and the line joining the mesial contact point and centroid of each tooth was measured and tabulated (representing the angle of rotation).

**Statistical analysis**
The mean area and angle of rotation of overlays produced by the four methods (hand tracing from study casts, hand tracing from wax impression method, radiopaque wax impression method, and xerographic method) were individually compared with the computerized technique using linear regression. The amounts of variation in the area and the angle of rotation of individual teeth bite marks were assessed using Mahalanobis distance by SPSS version 20 IBM Co-operation Switzerland.

**Results**
The overlays produced by the four methods (hand tracing from study casts, hand tracing from wax impression method, radiopaque wax impression method, and xerographic method) were individually compared with the computerized technique using linear regression. The amount of variation was then assessed using Mahalanobis distance.

The mean distance and standard deviation obtained from the measurements of tooth area for six anterior teeth in maxillary and mandibular arches were calculated [Table 1]. The mean value remained the same for all the methods (0.99), whereas the standard deviation for xerographic-based method was the least (1.46), followed by hand tracing from wax impression method (1.73) and hand tracing from study casts (1.18). The least significant was the radiopaque wax impression method (2.20) compared to other methods.

For angle of rotation [Table 2], the standard deviation for xerographic-based method (1.02) was least, followed by hand tracing from study casts (1.12) and radiopaque wax impression method (1.27), and the least significant was the hand tracing from wax impression method (1.48).

When comparing the overlay methods for area of the individual tooth [Table 1], xerographic method was the best showing least Mahalanobis distances in relation to the teeth 11, 21, 22, and overall. Wax impression method was the best for assessing 12, 23, 41, and 42. Radiopaque wax method was the best for assessing areas of 13, 31, and 32.
Table 1: Mahalanobis distance for each overlay method compared to computer-based method for area

| Tooth | Descriptives | Mahalanobis distance HT area | Mahalanobis distance WM area | Mahalanobis distance R area | Mahalanobis distance XM area | Best technique |
|-------|--------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------|
| 11    | n 30 30 30 30 | 2.7939 3.0374 3.3610 2.3051 | XM                          |
| 12    | n 30 30 30 30 | 0.6553 0.3638 0.6794 0.4771 | WM                          |
| 13    | n 30 30 30 30 | 0.9712 0.6746 0.4797 0.9379 | R                           |
| 21    | n 30 30 30 30 | 1.9712 2.9981 1.9756 1.7304 | XM                          |
| 22    | n 30 30 30 30 | 0.4279 0.4194 0.4867 0.3800 | XM                          |
| 23    | n 30 30 30 30 | 0.8392 0.3821 0.7293 0.4988 | WM                          |
| 31    | n 30 30 30 30 | 0.7978 0.4421 0.6625 1.3721 | R                           |
| 32    | n 30 30 30 30 | 0.5662 0.6708 0.3307 0.6183 | R                           |
| 33    | n 30 30 30 30 | 0.4649 0.8652 1.2488 0.5400 | HT                          |
| 41    | n 30 30 30 30 | 1.1065 0.6625 0.7477 1.2256 | WM                          |
| 42    | n 30 30 30 30 | 1.0336 1.8972 1.1159 0.9950 | WM                          |
Table 1: Contd...

| Tooth | Descriptives | Mahalanobis distance HT area | Mahalanobis distance WM area | Mahalanobis distance R area | Mahalanobis distance XM area | Best technique |
|-------|--------------|------------------------------|------------------------------|----------------------------|------------------------------|----------------|
|       | Mean         | 1.0491                       | 0.4650                       | 0.6335                     | 1.1651                       | WM |
|       | Std. deviation | 0.9889                       | 0.5754                       | 0.8268                     | 1.4736                       |                |
|       | Minimum      | 0.0002                       | 0.0018                       | 0.0004                     | 0.0000                       |                |
|       | Maximum      | 3.2992                       | 2.0851                       | 3.7558                     | 7.8653                       |                |
| 43    | n            | 30                           | 30                           | 30                         | 30                           |                |
|       | Mean         | 0.3235                       | 0.9856                       | 0.8532                     | 0.7161                       | HT |
|       | Std. deviation | 0.4992                       | 1.0010                       | 1.0432                     | 0.8599                       |                |
|       | Minimum      | 0.0000                       | 0.0053                       | 0.0025                     | 0.0157                       |                |
|       | Maximum      | 1.9013                       | 3.1655                       | 5.0913                     | 3.8703                       |                |
| Overall | n            | 360                          | 360                          | 360                        | 360                          |                |
|       | Mean         | 0.9972                       | 0.9972                       | 0.9972                     | 0.9972                       | NIL |
|       | Std. deviation | 1.8111                       | 1.7357                       | 2.2053                     | 1.4618                       | XM |
|       | Minimum      | 0.0000                       | 0.0000                       | 0.0000                     | 0.0000                       |                |
|       | Maximum      | 18.2563                      | 12.9555                      | 31.4412                    | 15.1545                      |                |

HT: Hand tracing from study casts; WM: Hand tracing from wax impressions of bite surface, R: Radiopaque wax impression method, XM: Xerographic-based method

Table 2: Mahalanobis distance for each overlay method compared to computer-based method for angle of rotation

| Tooth | Descriptives | Mahalanobis distance HT angle | Mahalanobis distance WM angle | Mahalanobis distance R angle | Mahalanobis distance XM angle | Best technique angle |
|-------|--------------|------------------------------|------------------------------|----------------------------|------------------------------|--------------------|
| 11    | n            | 30                           | 30                           | 30                         | 30                           | WM                 |
|       | Mean         | 1.0430                       | 0.9502                       | 1.0205                     | 1.2187                       | WM                 |
|       | Std. deviation | 0.4660                       | 0.4708                       | 0.4769                     | 0.6839                       |                |
|       | Minimum      | 0.1085                       | 0.0812                       | 0.0612                     | 0.0322                       |                |
|       | Maximum      | 1.7268                       | 1.6040                       | 1.9843                     | 3.9855                       |                |
| 12    | n            | 30                           | 30                           | 30                         | 30                           | WM                 |
|       | Mean         | 0.5418                       | 0.3698                       | 0.4538                     | 0.4905                       | WM                 |
|       | Std. deviation | 0.6223                       | 0.4246                       | 0.7376                     | 0.6416                       |                |
|       | Minimum      | 0.0003                       | 0.0029                       | 0.0019                     | 0.0015                       |                |
|       | Maximum      | 2.3456                       | 1.4115                       | 3.3905                     | 3.1934                       |                |
| 13    | n            | 30                           | 30                           | 30                         | 30                           | XM                 |
|       | Mean         | 1.9314                       | 1.9571                       | 2.0559                     | 1.6974                       | XM                 |
|       | Std. deviation | 1.3963                       | 3.1308                       | 1.7576                     | 1.0707                       |                |
|       | Minimum      | 0.1587                       | 0.1628                       | 0.1178                     | 0.0413                       |                |
|       | Maximum      | 5.4996                       | 17.6239                      | 8.2633                     | 4.5257                       |                |
| 21    | n            | 30                           | 30                           | 30                         | 30                           | HT                 |
|       | Mean         | 0.5323                       | 0.5943                       | 0.6031                     | 0.5516                       | HT                 |
|       | Std. deviation | 0.4355                       | 0.4957                       | 0.4156                     | 0.4477                       |                |
|       | Minimum      | 0.0030                       | 0.0001                       | 0.0001                     | 0.0002                       |                |
|       | Maximum      | 1.5143                       | 1.7616                       | 1.3967                     | 1.4266                       |                |
| 22    | n            | 30                           | 30                           | 30                         | 30                           | HT                 |
|       | Mean         | 0.3242                       | 0.3461                       | 0.5759                     | 0.3884                       | HT                 |
|       | Std. deviation | 0.4119                       | 0.3323                       | 0.5653                     | 0.3978                       |                |
|       | Minimum      | 0.0000                       | 0.0003                       | 0.0055                     | 0.0169                       |                |
|       | Maximum      | 1.8047                       | 1.2047                       | 2.0897                     | 1.4442                       |                |
| 23    | n            | 30                           | 30                           | 30                         | 30                           | XM                 |
|       | Mean         | 1.6324                       | 1.9043                       | 1.6163                     | 1.3899                       | XM                 |
|       | Std. deviation | 1.8572                       | 2.4007                       | 1.8411                     | 0.8128                       |                |
|       | Minimum      | 0.0003                       | 0.3551                       | 0.0710                     | 0.0239                       |                |
|       | Maximum      | 9.3211                       | 13.5021                      | 9.6200                     | 3.5903                       |                |
| 31    | n            | 30                           | 30                           | 30                         | 30                           | XM                 |
|       | Mean         | 1.4762                       | 1.4682                       | 1.7563                     | 1.2239                       | XM                 |

Contd...
Hand tracing from study casts was the best for assessing areas of 33 and 43.

For comparison/assessment of angle of rotation, xerographic-based method was the best for teeth 11, 12, 32, and 33; overall, wax impression method was the best for 11, 12, 32, and 33. Hand tracing from study casts was the best for teeth 21, 22, and 43, and radiopaque wax method was the best for teeth 41 and 42. There was no single best technique which showed least errors in overlay area and angle measurement as compared to the standard. Assessment of individual teeth showed that wax method was best suited for 4 out of 12 teeth both in area and angle assessment, which included mandibular central and lateral incisor and maxillary central incisor and canine. Assessment of angle for central incisor was better in wax method. Hand tracing and radiopaque wax methods were the least reliable showing higher distances from the standard.

Discussion

Bite marks have been defined by Mac Donald as “a mark caused by the teeth either alone or in combination with other mouth parts.” Human bite marks are most often found on the skin of victims or on food substances; while bite marks on food are usually well defined, the bite marks on skin are less defined. Bite marks can occur singly or at multiple sites, or may present as multiple bites at a single location. Each person has a unique dentition which can be replicated and helps in identifying the victim/or the culprit. Human bite marks have been described as elliptical or circular injuries.[5]

Bite marks can be analyzed using various techniques which could be either direct or indirect techniques. Direct technique involves the use of a model of the suspect’s teeth which is then compared to life-sized photographs of the bite mark, while indirect technique involves the use...
of transparent overlays, on which the biting edges of the suspect’s teeth are recorded. Transparent overlays can be produced by placing a sheet of acetate over the dental cast of the suspect’s teeth and tracing the biting edges with fine-tipped marker pen.[6]

Bite mark recording may be tricky in many tissue/food items. On skin/food substance, direct acetate tracing could be possible, whereas bite marks on curved surfaces may be radiographed following amalgam application. These could then be compared with original casts of the suspects.

Though there are various methods to determine human bite marks, according to Maloth,[1] xerographic analysis has been proved to be a better method. The present study aimed to evaluate the reliability and accuracy of the commonly used methods of human bite mark overlays, which included the hand tracing from study casts, hand tracing from wax impression, radiopaque wax impression method, xerographic-based method, and computer-based method. The computer-based method is more accurate, so this method was taken as the gold standard and other methods were compared with it to determine their accuracy.

Comparison of individual tooth area and angle assessment showed that there was considerable variation in the four techniques mentioned [Table 3]. Wax impression method was found to be a good technique to produce overlays of the suspect to compare the bite marks on food, skin, etc., The angle of rotation could be better assessed by wax method. As the wax method involved penetration of the teeth into wax, it allowed larger area of tooth to be exposed, which when traced was more accurate to correlate, as the line angles and contact points were better recorded in the wax impression.

There was considerable variation among the four overlay production methods in determination of incisal edge area [Figure 6]. It could be due to the subjective error that occurred while hand tracing. However, after statistical analysis, although wax impression method was found to be a good technique, xerographic overlay production method was found to be the most accurate method for determination of tooth area and angle of rotation among the four methods, despite the computer-based method being more reliable for bite mark analysis.

Xerographic method was the best among the four different methods to measure the area, followed by hand tracing from wax, hand tracing from study casts, and radiopaque wax impression method. Radiopaque wax impression method is not considered to be accurate because the area can increase with the depth of the bite on wax sheet which may alter based on the pressure applied.[1] On the other hand, magnification and distortion of radiographic image can also result in variation in measurements. Hand tracing from study casts is also not considered to be an accurate method as there could be subjective error while tracing.

Wax impression method may be better for recording the area and angle of tooth rotation of the teeth which are out of occlusion, The canines are the first teeth to contact the occlusal plane, and may hinder accurate recording of lateral incisor and maxillary first premolar in xerographic-based method and radiopaque wax impression method.

Advantage of xerographic method compared to other methods is that details like fracture on the model can be represented on the overlay, which cannot be accurately represented by hand tracing methods.[6]

In the present study, we found that xerographic method is more accurate and inexpensive, and can be used for preliminary screening purposes. Computer-based method is considered as a “gold standard” for bite mark analysis. However, further research on bite mark comparison is needed to enhance the reliability and accuracy of bite mark analysis. A database of computerized area and angulations can be formed for comparison with xerographic method.

**Conclusions**

The basis of using these analyses is that human teeth are unique and this asserted uniqueness is replicated on the
bitten substrate in sufficient detail to enable matching it with that of a single individual excluding all others.

Various comparison techniques are used in bite mark analysis and the choice of technique depends largely on personal preference. No single technique has been shown to be better than the others and very little research has been carried out to compare different methods. This study evaluated the accuracy of direct comparisons between suspect’s models and bite marks with indirect comparisons in the form of conventional traced overlays of suspects, and the xerographic technique was found to be the best.

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