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Potential and Limits of the Synergy between Cheiloscopy and Rugoscopy in Forensic Odontology.

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Abstract: Aim: The aim of this study is to evaluate the possibility of discriminating between the two sexes through the simultaneous use of cheiloscopy and palatine rugoscopy. **Materials and methods:** 80 palate and lip impressions (40 male and 40 female) were analyzed. The palate impressions were acquired using both analogue and digital methods. The lip impressions were first acquired analogically and then scanned. The morphology of the palatine rugae in the palate impressions was analyzed, with the exclusion of segmental rugae. The lip prints were analyzed in sextants and the morphology of the lip wrinkles was evaluated. **Results:** Palatine rugoscopy shows that women have more curvy rugae than men and that men have more rugae on the left side than on the right. Cheiloscopy gave no statistically significant results.
Discussion: Palatine rugoscopy showed some differences between the sexes, however, these results were not solid enough for reliable sex discrimination. Differences between the results of different studies can be attributed to ethnic and methodological variations. Cheiloscopy, on the other hand, did not provide statistically significant results for sex discrimination. **Conclusions:** Given the scant results obtained from palatine rugoscopy and the lack of statistically relevant results in cheiloscopy, together with a lack of concordance among the data found in literature, it is believed that it is not possible to discriminate between the two sexes by using the two methods contemporarily. Further research is needed.

Keywords: Cheiloscopy; Rugoscopy; Forensic dentistry; Sex

1. Introduction

Forensic odontology is a branch of dentistry that complements forensic medicine by offering its knowledge of the dental landscape. It is not simply a matter of mere notions for the purpose of assessing, in the event of litigation, who is right between the two parties, but also a set of skills that allow oral anatomical structures to be analyzed in order to attribute them to a certain individual, ethnic group or race when normal methods prove to be ineffective [1].

The two sexes have important anatomical differences (one need only think of the urogenital apparatus or the conformation of the pelvis [2]), and over the years there has been a focus on the possibility of discriminating between them through an analysis of the oral cavity, with all the limitations that this entails.

Oral structures designed for this purpose must be stable over time and difficult to alter. For example, dental elements are subject to wear and tear, and the pathologies that can affect them lead

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to their subsequent reconstruction, with the alteration of certain anatomical details such as grooves, ridges or dimples, which, naturally, are not reproduced to identically match the original state of the tooth.

In contrast, structures such as the hard palate [3] and the lips [4] are stable in time and are rarely altered, except in cases of oral surgery, for example in connective tissue sampling in the case of the palate or biopsies in both cases.

Several studies have investigated the possibility of discriminating between the two sexes by evaluating palatine rugoscopy or cheiloscopy. However, none of these studies appear to be exhaustive on sex identification and most focus only on one type of analysis.

It is the authors' opinion that the lack of comprehensiveness could be due to several factors, such as the efficiency of the classifications or the method used.

Palatine rugae are anatomical structures that are unique to each individual [5], consisting of a large proportion of connective tissue covered by the keratinized tissue present in the hard palate. They are studied in forensic odontology by means of palatine rugoscopy by assessing their number, shape and size [6].

Cheiloscopy, on the other hand, deals with the study of labial impressions, which, like palatine rugae, are stable over time and have a pattern unique to each individual [6].

This study aims to discriminate the two sexes on the basis of the morphology of the palatine rugae and labial wrinkles using palatine rugoscopy and cheiloscopy, respectively.

2. Materials and Methods

In this study, a total of 90 volunteers were recruited: 49 men and 41 women. Two distinct types of samples were collected from each participant: palate impressions and lip prints. These samples were obtained following specific protocols, described below, to ensure the accuracy and reproducibility of the data. To ensure the integrity of the results and minimize possible errors or bias from invalid samples, the following inclusion and exclusion criteria were established for sample analysis:

Inclusion Criteria:

- Known gender;
- Presence of both impressions (palatal and labial);
- Stable general health condition;
- Absence of previous allergic reactions to lipsticks;
- Belonging to the Caucasian ethnic group.

Exclusion Criteria:

- Refusal of informed consent;
- Poor quality of one or both impressions;
- Anatomical abnormalities of the lips and/or palate (e.g., scars or clefts);
- Presence of foreign objects interfering with impression taking (e.g., lip piercings or palatal expanders).

2.1. Palatine Rugoscopy

After obtaining informed consent from the volunteers, each subject underwent a thorough clinical examination of the palate to detect any abnormalities that could exclude them from the study. Palatine impressions were collected following two methodologies: 40 impressions were taken in the traditional way using alginate and cast with type IV plaster, then scanned with a laboratory scanner; the remaining 50 impressions were scanned directly with an iTero intraoral scanner (Align Technology, Risch-Rotkreuz, Switzerland) following the manufacturer's instructions. A unique identification number was marked on each impression, while relevant information, such as the age and sex of each subject, was noted in a separate document. Once all the impressions had been acquired and the invalid ones eliminated, the palatine rugae were analyzed according to the Thomas and Kotze classification [7] but evaluating only their morphology and not their size. Rugae that were too small (≤ 3 mm) and could be attributable to segmental ones were discarded:

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- Converging;
- Diverging;
- Straight;
- Curvy;
- Circular;
- Wavy.

The data from palatine rugoscopy were entered into Microsoft Excel 2021 software (Microsoft Corporation) and subdivided by subject, gender and palate. The sum, mode, rugae mean and P-value were calculated with the same software using the Data Analysis (Regression) tool.

2.2. Cheiloscopy

Like with rugoscopy, each subject underwent a preliminary clinical examination of the lips. Subsequently, the lips were moistened and carefully cleaned to ensure optimal conditions. Using disposable cotton buds to avoid contamination (e.g., HSV), a thin layer of red liquid lipstick was applied to the participants' lips, who then rubbed their lips together to distribute it evenly. The impressions of the upper and lower lips were simultaneously captured on strips of transparent adhesive tape and transferred to blank cards for easy analysis and handling.

Again, a unique identification number was assigned to each impression, the same as for the palatal impressions. The impressions were digitized and optimized using Adobe® Photoshop. In each sextant, the predominant pattern was identified according to the Tsuchiashi classification [8]:

- Type 1: complete vertical grooves;
- Type 1': partial vertical grooves;
- Type 2: branched grooves;
- Type 3: intersected grooves;
- Type 4: reticular grooves;
- Type 5: undetermined grooves.

The data collected through cheiloscopy were also entered into Microsoft Excel 2021 software and grouped into tables by subject, sex and type of analysis. Mode, sum, mean by sextant and sex, and P-value were calculated with the same software using the Data Analysis (Regression) tool.

2.3. Data Analysis and Review

Palatine rugoscopy and cheiloscopy were performed by two different operators respectively, and the results obtained were reviewed by both to ensure data consistency. Subsequently, the statistical results were analyzed for possible correlations between the patterns found and the sex of the subjects, with the aim of developing sex discrimination methods based on these morphological features.

3. Results

Of the samples collected from the 90 volunteers, the impressions of 10 subjects (1 man and 9 women) were discarded, leaving 40 men and 40 women. The average age of the participants was 28.9 years (26.92 for men and 30.05 for women).

3.1. Palatine Rugoscopy

The mode analysis [Table 1] of the male group shows that, in the male group, on both the right and the left side, there are typically 3 rugae, giving a total of six rugae per palate. In the female group on the right side there can be 3 or 4 rugae, while on the left side 3. In this case, the mode value for the total number of rugae is 7. As for the single type of rugae, the male group shows a value of 1 for diverging and wavy rugae on each side for a total of 2 diverging rugae and two 2 wavy rugae per impression taken. Again, in the male group, a mode value of 1 for straight rugae is observed on the left side but not on the right, where the value is equal to 0. The total of straight rugae has a value of 2 for each impression. In the female group, an identical situation to the male group was observed

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with regard to diverging rugae. However, unlike the male group, there was a mode value of 0 for straight rugae on the right and 1 and 0 on the left, with a mode value of 1 for each impression taken. The wavy rugae in the female group have a mode of 1 on the left side and 0 on the right side. The total of wavy rugae gives a mode of 1. Curvy rugae have a mode value of 0 and 1 on the left side and equal to 1 on the right side. The total of curvy rugae has a mode value of 1, while in the male group there is always a value of 0.

Table 1. Mode value of Palatal Rugae by Palate Side and Sex.

Mode Value of Palatal Rugae												
Male							**Female**					
Right	3	**Left**	3	**Tot.**	6		**Right**	3;4	**Left**	3	**Tot.**	7
Converging	0	Converging	0	Converging	0		Converging	0	Converging	0	Converging	0
Diverging	1	Diverging	1	Diverging	2		Diverging	1	Diverging	1	Diverging	2
Straight	0	Straight	1	Straight	2		Straight	0	Straight	0;1	Straight	1
Curvy	0	Curve	0	Curvy	0		Curvy	0;1	Curvy	1	Curvy	1
Circular	0	Circular	0	Circular	0		Circular	0	Circular	0	Circular	0
Wavy	1	Wavy	1	Wavy	2		Wavy	0	Wavy	1	Wavy	1

The results obtained on the total number of rugae [Table 2] and their mean [Table 3] show a total of 271 palatine rugae (mean of 6.78 per sample) for the men's group and 273 palatine rugae (mean of 6.83 per sample) for the women's group, determining no statistically significant difference in the total number of rugae between men and women ($P>0.05$). Evaluating the distribution of rugae between men and women on the right and left side, the results are also similar and the statistical analysis again gives a statistically non-significant result ($P>0.05$). When the differences between the two halves of the palate within a single group are considered, there is a statistically significant difference between right and left in the men's group ($P=0.02$) but not in the women's group ($P>0.05$) [Table 5].

Table 2. Total Palatal Rugae by Side and Sex.

Total Palatal Rugae												
Male							**Female**					
Right	132	**Left**	139	**Tot.**	271		**Right**	137	**Left**	136	**Tot.**	273
Convergin g	3	Convergin g	9	Convergin g	12		Convergin g	4	Convergin g	3	Convergin g	7
Diverging	37	Diverging	33	Diverging	70		Diverging	33	Diverging	21	Diverging	54
Straight	39	Straight	32	Straight	71		Straight	41	Straight	38	Straight	79
Curvy	15	Curvy	24	Curvy	39		Curvy	26	Curvy	27	Curvy	53
Circular	1	Circular	6	Circular	7		Circular	2	Circular	5	Circular	7
Wavy	37	Wavy	35	Wavy	72		Wavy	31	Wavy	42	Wavy	73

Table 3. Average Palatal Rugae.

Average Palatal Rugae												
Male							**Female**					
Right	3,30	**Left**	3,48	**Tot.**	6,78		**Right**	3,43	**Left**	3,40	**Tot.**	6,83

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Converging	0,08	Converging	0,23	Converging	0,30		Converging	0,10	Converging	0,08	Converging	0,18
Diverging	0,93	Diverging	0,83	Diverging	1,75		Diverging	0,83	Diverging	0,53	Diverging	1,35
Straight	0,98	Straight	0,80	Straight	1,78		Straight	1,03	Straight	0,95	Straight	1,98
Curvy	0,38	Curvy	0,60	Curvy	0,98		Curvy	0,65	Curvy	0,68	Curvy	1,33
Circular	0,03	Circular	0,15	Circular	0,18		Circular	0,05	Circular	0,13	Circular	0,18
Wavy	0,93	Wavy	0,88	Wavy	1,80		Wavy	0,78	Wavy	1,05	Wavy	1,83

No statistically significant results were found within the individual groups (P>0.05) when assessing rugae morphology. A comparison between the individual rugae morphologies in the two groups [Table 4] shows that the female group tends to have a higher frequency of curvy rugae with respect to the male group (P=0.04). However, no other statistically significant results between the two sexes were found for the remaining rugae types (P>0.05).

Table 4. P-value of the morphology of Palatine Rugae M/F, total, right and left side.

P-value of the comparison between the two sexes			
	Total Type Rugae M/F	**Total Type Rugae R M/F**	**Total Type Rugae L M/F**
Converging	0,95	0,56	0,49
Diverging	0,75	0,57	0,52
Straight	0,38	1,00	0,71
Curvy	0,04	0,64	0,06
Circular	0,81	0,82	0,75
Wavy	0,22	0,19	0,84

Table 5. Difference between the two groups.

Difference between groups	
Comparison	**P-value**
Right M vs F	0,37
Left M vs F	0,69
Right vs Left M	0,02
Right vs Left F	0,23

3.2. Cheiloscopy

The mode results in the two groups [Table 6] show differences only for the second sextant, with type 1′ in the male group and type 4 in the female group.

Table 6. Mode morphology Labial Rugae per sextant.

Mode Cheiloscopy per Sextant					
Male			**Female**		
I Sext.	II Sext.	III Sext.	I Sext.	II Sext.	III Sext.

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1'	1'	1'	1'	4	1'
VI Sext.	V Sext.	IV Sext.	VI Sext.	V Sext.	IV Sext.
2	4	2	2	4	2

The mean appears to be homogeneous [Table 7]. Overall, no significant differences are noted when comparing types between the two groups [Table 8].

Table 7. Average Labial Rugae per sextant.

Type Average per Sexants											
Male			**Female**			**Male**			**Female**		
I Sextant						**IV Sextant**					
Type	Average	Tot.	**Type**	Average	Tot.	**Type**	Average	Tot.	**Type**	Average	Tot.
1	0,18	7	**1**	0,20	8	**1**	0,15	6	**1**	0,13	5
1′	0,30	12	**1′**	0,40	16	**1′**	0,23	9	**1′**	0,18	7
2	0,15	6	**2**	0,23	9	**2**	0,25	10	**2**	0,35	14
3	0,15	6	**3**	0,05	2	**3**	0,05	2	**3**	0,13	5
4	0,05	2	**4**	0,13	5	**4**	0,15	6	**4**	0,10	4
5	0,18	7	**5**	0,00	0	**5**	0,18	7	**5**	0,13	5
II Sextant						**V Sextant**					
Type	Average	Tot.	**Type**	Average	Tot.	**Type**	Average	Tot.	**Type**	Average	Tot.
1	0,18	7	**1**	0,10	4	**1**	0,25	10	**1**	0,23	9
1′	0,23	9	**1′**	0,15	6	**1′**	0,08	3	**1′**	0,13	5
2	0,20	8	**2**	0,03	1	**2**	0,10	4	**2**	0,08	3
3	0,18	7	**3**	0,08	3	**3**	0,15	6	**3**	0,13	5
4	0,15	6	**4**	0,58	23	**4**	0,33	13	**4**	0,35	14
5	0,08	3	**5**	0,08	3	**5**	0,10	4	**5**	0,10	4
III Sextant						**VI Sextant**					
Type	Average	Tot.	**Type**	Average	Tot.	**Type**	Average	Tot.	**Type**	Average	Tot.
1	0,20	8	**1**	0,18	7	**1**	0,15	6	**1**	0,20	8
1′	0,25	10	**1′**	0,30	12	**1′**	0,28	11	**1′**	0,20	8
2	0,18	7	**2**	0,23	9	**2**	0,28	11	**2**	0,30	12
3	0,15	6	**3**	0,15	6	**3**	0,18	7	**3**	0,10	4
4	0,13	5	**4**	0,13	5	**4**	0,03	1	**4**	0,13	5
5	0,10	4	**5**	0,03	1	**5**	0,10	4	**5**	0,08	3

Table 8. Average labial rugae by type.

Average per Type								
Male			**Female**			**P-value**		
Type	**Average**	**Tot.**	**Type**	**Average**	**Tot.**	**Type**	**P**	
1	0,18	44	**1**	0,17	41	**1**	0,41	
1′	0,23	54	**1′**	0,23	54	**1′**	0,69	
2	0,19	46	**2**	0,20	48	**2**	0,88	
3	0,14	34	**3**	0,10	25	**3**	0,44	
4	0,14	33	**4**	0,23	56	**4**	0,82	
5	0,12	29	**5**	0,07	16	**5**	0,40	

The only statistically significant differences are observed within the individual groups. When comparing the various types within the individual groups, the most statistically significant differences are observed in the men's group [Table 9] where type 1′ and 2 are those with the most statistically significant differences, 3, while type 3 and 4 show 2 statistically significant differences. In

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the women's group, few statistically significant differences are observed compared to the men's group. It can be seen that type 1′ and 4 present the most differences, while type 2 and type 5 show no statistically significant differences compared to the other types in the same group [Table 10].

Table 9. P-value Male group.

Male Type P-value						
-	**Type 1**	**Type 1′**	**Type 2**	**Type 3**	**Type 4**	**Type 5**
Type 1	-	0,08	0,33	0,29	0,98	0,05
Type 1′	0,08	-	0,04	0,05	0,05	0,4
Type 2	0,33	0,04	-	0,04	0,05	0,23
Type 3	0,29	0,05	0,04	-	0,6	0,96
Type 4	0,98	0,05	0,05	0,6	-	0,77
Type 5	0,05	0,4	0,23	0,96	0,77	-
Tot. <0,05	1	3	3	2	2	1

Table 10. P-value Female group.

Female Type P-value						
-	**Type 1**	**Type 1′**	**Type 2**	**Type 3**	**Type 4**	**Type 5**
Type 1	-	0,37	0,06	0,68	0,03	0,47
Type 1′	0,37	-	0,27	0	0,02	0,95
Type 2	0,06	0,27	-	0,67	0,13	0,08
Type 3	0,68	0	0,67	-	0,37	1
Type 4	0,03	0,02	0,13	0,37	-	0,14
Type 5	0,47	0,95	0,08	1	0,14	-
Tot. <0,05	1	2	0	1	2	0

4. Discussion

The aim of this study was to identify morphological differences between the sexes in palatine rugae and labial rugae. Through a comparison of our results with those found in literature, numerous differences can be observed.

With regard to palatine rugoscopy, substantial differences can probably be attributed to variations between populations, as shown in two studies based on different ethnicities [9,10]. Furthermore, not all studies use the Thomas and Kotze classification.

A study on the Iranian population found that the most common pattern of palatine rugae is straight, followed by wavy and curvy, without distinction between the sexes [11]. In our study, the straight pattern was the one that was most present in both sexes, with no statistically significant differences. However, the diverging pattern was seen to be more common than the curvy one, which was still more frequent in women than men but without statistically significant differences.

A further study on the Tibetan and Indian population identified the diverging pattern to be the most common [12]. Similarly, a study on Iranian children found no difference in the total number of rugae between the sexes, but indicated a greater presence of rugae on the right side in the female group and a predominance of the curvy pattern in men, in contrast with our results [13].

The data collected in our sample confirm what has been reported with regard to a population in Kerala, namely that the circular pattern is rare [14]. However, in our study, we observed no significant differences between men and women. Similar to a study on the Central Indian population, we found

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that men tend to have more wavy rugae and women more straight rugae, although these differences were not statistically significant in our study [15].

In a study on the Maharashtrian population, the wavy pattern prevailed in males, but this was a very small population [16]. In concordance with a study on the Dravidian population, we observed more curvy rugae in women than in men, with a statistically significant result [17].

A study on children in Davangere showed differences between men and women in converging and diverging patterns. These differences were not statistically significant in our study [18]. However, the curvy pattern was more frequent in men, a situation not observed in our data, where the total number of curvy rugae was greater in the female group.

In a study on 100 Sudanese, a higher frequency of converging rugae on the left side of the palate was observed in males, a finding also observed in our study [19]. A study on the Mediterranean population found no statistically significant differences between the groups [20], while a study on five different Indian populations found significant differences in the number of rugae between the right and left side in the female group [21]. In our study, this difference was observed in the male group with statistical significance. In contrast, significant differences were found between the male group and the female group in circular and converging rugae in 100 subjects from Meerut [22].

In general, with palatine rugoscopy, we note how difficult it is to obtain concrete data capable of discriminating between the two sexes, probably due to the limited size of the samples studied. Some studies have proposed differential functions, such as a population study in coastal Andhra [23]. One study revealed similarities between relatives, suggesting a certain heritability of palatine rugae patterns [24].

The results obtained in our study on palatine rugoscopy, although based only on the shape of the rugae and not on their size, determine that there are differences in the number of curvy rugae between the two sexes and that there are differences between the total number of male rugae on the right and left side, but these differences were not found in the female group. The only statistically significant results obtained in rugoscopy do not allow discrimination between the two sexes as it can correctly discriminate sex only in a few individuals. However, if there were to be a database, it could be used in rare cases to identify an unrecognizable subject [25].

Even in the field of cheiloscopy, data are not always in agreement. On the one hand, this may be attributable, in part, to variations between populations, but on the other hand, several discrepancies arise from the numerous methods used in literature [26] and the difficult interpretation of the data collected, where operator-dependent errors may be frequent [31].

Unlike in rugoscopy, where the methods used are analogue, with alginate impressions, and digital, with the aid of intraoral scanners, in cheiloscopy we can photograph the subject's lips directly, use a non-porous surface (e.g., a mirror) to photograph latent impressions, apply lipstick or other transfer medium and press the lips onto paper or adhesive tape, press the lips onto a suitable surface and develop impressions with fingerprint powder or magnetic powder [27,28]. There is an incalculable margin of error that derives from the methodology chosen [29], both with regard to the different materials that can be used [30], and the difficulty of taking impressions that present all the lip lines intact, without smears and easily readable [31]. Furthermore, the operator-dependent error derived from the analysis of the impressions themselves must also be taken into account [32].

The literature reports extremely discordant results concerning the possible use of cheiloscopy to determine sex, and the articles that have found statistically significant differences do not always agree with each other.

In a study of 2112 individuals from the population of Calicut, Kenya, a predominance of Type 1 and 1′ was found in male subjects and Type 4 and 5 in female subjects [33], a difference partially observed in our study and limited to the second sextant, where Type 1 was found to be predominant in men and Type 4 in women. This predominance of Type 4 in the female gender has also been observed in other studies [34,35].

A study of 100 students at the Shri Sathya Sai Medical College and Research Centre also divided the lip prints into sextants and analyzed the samples, finding statistically significant differences at

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sextants 1, 3, 4 and 6 [36], the exact opposite of the results in our study, which show sex-related differences only in the second sextant, although these were not statistically significant.

Another study of 600 individuals from rural and urban locations in Aurangabad, Maharashtra, India, observed that in the upper lip, Type 4 was more common in the lateral segments of females than males, while Type I was more common in the lateral segments of males than females, resulting in statistically significant results between the sexes in the lateral segments [37], while this difference was not significant in the medial segments, a situation again not observed in our study.

Because of the data obtained in our study, which show the absence of statistically significant results, and because of the great heterogeneity of the results found in literature, we believe that cheiloscopy is not a reliable approach for determining sex. Even two recent systematic reviews report discordant opinions. The first [26] agrees with us regarding the problems of methodology and considers cheiloscopy invalid for determining the sex of a subject; the second, which analyses both rugoscopy and cheiloscopy, finds the method even more reliable than rugoscopy, with an accuracy of 80%, [38].

Despite this, we consider cheiloscopy to be a valid tool for 1-1 matching, e.g., in the field of criminology if a latent print can be found, as lip prints are unique and temporally stable [8,39–43].

In the light of the results observed in the literature, the effectiveness of both palatine rugoscopy and cheiloscopy is poor. The data obtained on small populations certainly make an anthropological contribution but do not allow the two sexes to be discriminated effectively, even within the same populations.

We therefore suggest that although studies on small populations may have statistically valid results on anatomical traits of palatine rugae and labial rugae, they do not allow effective discrimination between sexes, even within the same populations.

On the basis of our results and on the comparison with the various studies in literature, we can state that, despite some differences in the number of curvy rugae between the sexes and between the sides of the palate in men, these are not sufficient for effective sex discrimination. The data obtained in our study indicate a poor potential of palatine rugoscopy and a total ineffectiveness of cheiloscopy in discriminating between sexes. The combined use of palatine rugoscopy and cheiloscopy, therefore, did not provide statistically significant results sufficient to discriminate the sexes efficiently..

5. Conclusions

From the results obtained in this study, palatine rugoscopy does not show many differences between the two sexes. As for cheiloscopy, the results obtained from the comparison between the two groups were all statistically non-significant. The results obtained in palatine rugoscopy do not allow one of the two sexes to be identified given that statistical significances only concern the curvy pattern. The lack of statistically significant results even in only one of the two methods used, in our case cheiloscopy, determines that palatine rugoscopy and cheiloscopy applied simultaneously cannot discriminate between the two sexes and that the data collected in this analysis can only serve as an anthropological analysis and not be applied in forensic odontology. Further research is needed to confirm the findings of this study.

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