Potential and limits of the synergy between cheiloscopy and rugoscopy in forensic odontology

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Abstract

This study aims to assess the possibility of sex discrimination through the combined use of cheiloscopy and palatine rugoscopy. Eighty palate and lip impressions (40 male and 40 female) were analyzed. Palate impressions were acquired both analogically and digitally, while lip impressions were initially analogical and then scanned. Palatine rugae morphology was analyzed, excluding segmental rugae, and lip wrinkles were evaluated in sextants. Palatine rugoscopy did not reveal statistically significant differences between men and women. Cheiloscopy showed no significant differences between the sexes but some within individual sexes. Palatine rugoscopy results did not show sexual dimorphism. Variations in results across studies could be attributed to ethnic or methodological differences. Cheiloscopy failed to produce statistically significant differences between sexes but did show differences within the same sex. Given the lack of significant results in palatine rugoscopy and cheiloscopy, as well as inconsistencies in the literature, it appears that sex discrimination through the simultaneous use of these methods is not feasible. Further research is recommended.

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Introduction

Forensic odontology, a specialized field within dentistry, plays a crucial role in forensic science by leveraging dental expertise to support legal investigations. This discipline goes beyond resolving disputes between parties; it encompasses a comprehensive set of techniques for analyzing dental and oral structures. These analyses are instrumental in identifying individuals or associating remains with specific ethnic groups, particularly when conventional identification methods are inadequate.[1]

The two sexes have important anatomical differences (in addition to 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 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 like the hard palate[3] and lips[4] remain largely unchanged over time, with minimal alterations occurring only in specific circumstances, such as oral surgeries. Examples include connective tissue sampling from the palate or biopsies involving either the palate or lips.

Several studies have investigated the possibility of discriminat-

ing 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 distinct anatomical features found in the hard palate, primarily composed of connective tissue and overlaid by keratinized epithelium.[5] In forensic odontology, these structures are analyzed through palatine rugoscopy, which focuses on evaluating their number, form, and dimensions.[6] Conversely, cheiloscopy involves examining lip prints, which, similar to palatine rugae, exhibit unique patterns for each individual and remain consistent over time.[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.

Materials and Methods

In this study, a total of 90 volunteers were recruited: 49 men and 41 women. The volunteers consisted of students from the University of Palermo and patients from private practices. They underwent examinations in two different Italian regions (Sicily and Abruzzo) by four different operators from November 2023 to January 2024. 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).

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] (Figure 1) but evaluating only their morphology and not their size. Palatal rugae were classified according to Thomas and Kotze intosix morphological patterns: straight, curvy, wavy, circular, converging, and diverging.

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. herpes simplex virus), 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 Tsuchihashi classification[8] (Figure 2): 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.

Figure 1. Thomas and Kotze palatine rugae classification. Image obtained from a scan performed with an iTero device.

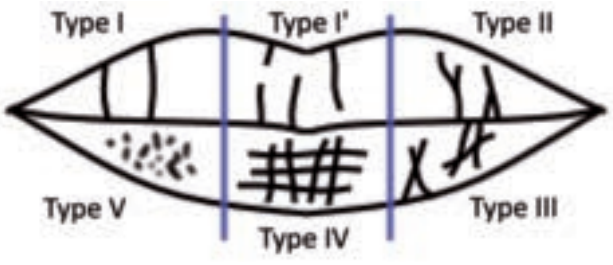

Figure 2. Tsuchihashi[8] lip grooves classification.

Data analysis and review

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

The data from palatine rugoscopy and cheiloscopy were entered into Microsoft Excel 2021 software (Microsoft Corporation) and subdivided by subject and gender. The sum, mode and means of rugae and grooves were calculated with the same software. The statistical difference, p value, of total number of palatal rugae and type of labial groovers was analyzed with a χ^2 test, using the function TEST.CHI.QUAD after creating the contingency tables (made up of 2 columns for the groups and 40 rows for the subjects) for comparing the number of palatal rugae or type of lip pattern. Furthermore, given the presence of values equal to 0, a pseudocount of 0.01 was applied to the values obtained from the subjects to make the calculation of the $\chi 2$ test applicable. The power analysis (1-β) post hoc was performed with G*power software (University of Kent, Canterbury, Kent, UK) using a Cohen's index (*w*), as effect size, for each statistically significant result obtained to evaluate them further, a significance level of 0.05 and 39 degrees of freedom. A post-hoc analysis was preferred as it enjoys greater flexibility than an a priori analysis, even if subject to type I errors; furthermore, the choice was conditioned by the number of subjects. Mode and average have a simple descriptive value.

Results

Among 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 10 impressions were excluded due to poor quality prints of palatal rugae or lip prints that did not meet the study's inclusion criteria. The average age of the participants was 28.9 years (26.92 for men and 30.05 for women).

Palatine rugoscopy

In the context of palatal impressions, no significant differences were observed in identifying the pattern of palatal rugae; the 40 digital impressions accurately mirrored the analog alginate impressions. The mode analysis (Table 1) of the male group shows that, 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 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 mode value of 2 for each impression. In the female group, an identical situation to the male group was observed with regard to diverging rugae. However, unlike the male group, there was a mode value of 0 for straight rugae on the right side 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).

The results obtained on the total number of rugae and their mean show a total of 271 palatine rugae (mean number of rugae: males 6.78±1.27, females 6.70±1.10) 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 not a statistically significant difference between right and left in both men's and women's group ($p>0.05$) (Table 2).

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

Table 2. 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	1	1	1
Diverging	0.96	0.99	0.98
Straight	0.65	0.65	0.94
Curvy	0.98	0.98	1
Circular	1	1	1
Wavy	0.65	0.65	0.95

M/F, male/female; R, right; L, left.

No statistically significant results were found within the individual groups (p>0.05) when assessing rugae morphology. No statistically significant results between the two sexes were found for the rugae types (p>0.05).

Cheiloscopy

The mode results in the two groups show differences only for the second sextant, with type 1' in the male group and type 4 in the female group.

The mean values appeared homogeneous between the two groups (p>0.05). (Table 3).

The only statistically significant differences are observed within the individual groups. When comparing the various types within the individual groups, the most significant statistically differences are observed in the men's group (Table 4). In the women's group, few statistically significant differences are observed compared to the men's group (Table 5).

Power analysis

The power analysis was carried out with statistically significant results. The Cohen's Index w ranged from 0.874 to 0.910, the value of 1-β from 0.99 to 1 (Table 6).

Table 3. Average labial rugae by type.

Average labial rugae by type					
Type	Male Average	Tot.	Type	Female Average	Tot.
1	0.18	44	1	0.17	41
1'	0.23	54	1'	0.23	54
2	0.19	46	2	0.20	48
3	0.14	34	3	0.10	25
4	0.14	33	4	0.23	56
5	0.12	29	5	0.07	16

Table 4. P value male group.

Male type p value						
-	Type 1	Type 1'	Type 2	Type 3	Type 4	Type 5
Type 1	-	0.01	0.02	>0.05	>0.05	0.05
Type 1'	0.01	-	0.00	>0.05	>0.05	>0.05
Type 2	0.02	0.00	-	0.01	0.03	>0.05
Type 3	>0.05	>0.05	0.01	-	>0.05	>0.05
Type 4	>0.05	>0.05	0.03	>0.05	-	>0.05
Type 5	0.05	>0.05	0>0.05	>0.05	>0.05	-
Tot. <0.05	3	2	4	1	1	1

Table 5. P value female group.

Female type p value						
-	Type 1	Type 1'	Type 2	Type 3	Type 4	Type 5
Type 1	-	>0.05	>0.05	>0.05	0.05	>0.05
Type 1'	>0.05	-	>0.05	>0.05	0.04	>0.05
Type 2	>0.05	>0.05	-	>0.05	>0.05	>0.05
Type 3	>0.05	>0.05	>0.05	-	>0.05	>0.05
Type 4	0.05	0.04	>0.05	>0.05	-	>0.05
Type 5	>0.05	>0.05	>0.05	>0.05	>0.05	-
Tot. <0.05	1	1	0	0	2	0

Table 6. Power analysis.

Power analysis				
Group	Comparison type	X^2	*w*	1-β
Male	1-1'	62.13	0.88	0.999
Male	1-2	58.69	0.86	0.997
Male	1-5	54.75	0.83	0.995
Male	1'-2	66.31	0.91	0.999
Male	2-3	61.05	0.87	0.998
Male	2-4	57.87	0.85	0.997
Female	1-4	54.98	0.83	0.995
Female	1'-4	55.72	0.84	0.996

Discussion

The aim of this study was to identify the morphological differences between the sexes in palatal rugae and labial rugae. Through a comparison of our results with those found in the 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.

It is important to underline that forensic odontology and its methods are constantly evolving, particularly in the field of palatal rugoscopy. In addition to traditional methods such as image superposition, more complex methods are being utilized to determine the shape of palatal rugae. 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 populations 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] but without statistically significant differences.

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 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, without a statistically significant result.[17]

A study on children in Davangere, India, 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 subjects, a higher frequency of converging rugae on the left side of the palate was observed in males,[19] a finding also observed in our study but without statistically significant differences. 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 without 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 the challenges in obtaining definitive data capable of distinguishing between sexes, likely due to the limited sample sizes studied. However, others describe palatal rugoscopy as a relatively valid and additional tool in forensic analysis. Some studies have proposed differentiating functions, such as a population study in coastal Andhra Pradesh, India.[23] Additionally, one study revealed similarities among 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 no differences in the two groups. The lack of statistically significant results obtained in rugoscopy do not allow a discrimination between the two sexes. A structured database of palatal rugae could still be useful in rare cases for identifying an unrecognizable individual.[25]

In the field of cheiloscopy, consensus is not always achieved. This can partly be attributed to variations among different populations. However, a significant portion of discrepancies arises from the diverse methodologies employed in the literature,[26] coupled with the complex interpretation of collected data, where operator-dependent errors are common. Unlike rugoscopy, which utilizes both analog methods, such as alginate impressions, and digital techniques with intraoral scanners, cheiloscopy involves directly photographing the subject's lips or capturing latent impressions on a nonporous surface (e.g., a mirror). Methods may also include applying lipstick or another transfer medium to press the lips onto paper or adhesive tape, or using fingerprint or magnetic powder to develop impressions from a suitable surface.[27,28] The potential for error is considerable, stemming from both the choice of methodology and the inherent difficulty in capturing clear, intact lip impressions without smudges. Additionally, operator-dependent errors in the analysis of lip impressions must also be considered, as described by Paušić *et al*.[29]

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.

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 cheiloscopy even more reliable than rugoscopy, with an accuracy of 80%.[30]

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]

In light of the results observed in the literature, both palatine rugoscopy and cheiloscopy exhibit limited effectiveness. The data obtained from small populations certainly contribute to anthropology but do not effectively distinguish between sexes, even within the same populations. Our study focused on a Mediterranean population. It is essential to consider that Italy's history includes various occupations that have significantly influenced the genetic diversity of its population. 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 in comparison with the various studies in literature, we can state that the data obtained in this study are not sufficient for effective sex discrimination by both platine rugoscopy and cheiloscopy. The combined use of palatine rugoscopy and cheiloscopy, therefore, did not provide statistically significant results sufficient to discriminate the sexes efficiently.

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 and the differences within individual groups alone in cheiloscopy do not allow discrimination between the two sexes. The lack of statistically significant results even in only one of the two methods used, in our case both, 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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