ORIGINAL ARTICLE

Assessment of cheiloscopy in sex determination using lysochrome - A preliminary study

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Abstract

Introduction: The present study was undertaken with the objective of ascertaining whether latent lip prints generated by persistent lipsticks and developed using lysochrome dyes have the potential of use in sex determination and personal identification. **Materials and Methods:** This study included a total of 100 subjects (50 males and 50 females) whose latent lip prints were obtained by applying the persistent lipstick Revlon ColorStay Overtime® manufactured by Revlon® consumer products corporation, NewYork, USA, and lifting the prints with cellophane sheets. The prints were then developed using lysochrome dyes, and all the samples were blinded and then graded based on defined patterns from the Suzuki and Tsuchihashi classification. **Results:** No two lip prints were found to be alike. Type I was found to be the most prevalent type. In the female population, Type I (61%) was most prevalent, followed by Type I' (28%), Type II (9%), Type III (2%), Type IV (1%), and Type V (1%); in the male population, Type I (33%) was most prevalent, followed by Type II (23%), Type III (18%), Type IV (14%), Type I' (10%), and Type V (3%). Two examiners were able to determine the correct sexes from the given sample sizes. Their interobserver agreement was assessed using the kappa coefficient for males (κ =0.870) and females (κ = 0.870). Their accuracy was assessed with a confidence interval (CI) of 91.48-99.38. **Conclusion:** Lysochrome dyes are very efficacious in developing latent lip prints. This preliminary study has conclusively proved that latent lip prints developed with lysochrome dyes hold the potential for use in sex determination and can be maintained in a digital database.

Key words: Cheiloscopy, forensic odontology, lip prints, lysochrome dye persistent lip sticks, sex determination

Introduction

Establishing the identity of an individual, in a forensic context, through sex determination is of great ethical and humanitarian use. They allow legal investigations, inquests, and other tribunals, such as those held by coroners, medical examiners, judges, and accident enquiries to proceed with firm knowledge about the identity of an individual. Recent research has proved that lip prints, the study of which is referred to as cheiloscopy (*cheilos* meaning "lip" and *skopein* meaning "to observe or examine," in Greek) is a technique of invaluable use in personal identification.[1] Although previous research has conclusively proved that lip prints hold potential for use in sex determination and personal identification, our search of the literature has revealed an extreme scarcity of research on the potential for sex determination held by latent lip prints. The lip prints

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produced by traditional lipsticks are generally easily visible and identifiable.[2] However, in real-life crime scenes they are rarely visible, whereas latent (invisible) lip prints may be more prevalent and may present as hidden evidence. These latent lip prints can be processed using special reagents and dyes, such as lysochrome dyes.[3]

Lysochrome is a generic term for compounds that have the ability to dye fatty acids. A lysochrome molecule contains a portion that dissolves in contact with fat ("lyso") and another that is responsible for color ("chrome"). The use of persistent lipsticks is highly prevalent now and they, being nontransferable in nature, leave latent lip prints. Lysochrome can be applied in the development of latent lip prints generated by longstanding persistent lipsticks. The lip prints, once developed, can be used for the sex determination and personal identification of individuals.[4] The most widely accepted classification regarding the morphological variations in lip print pattern was given by Suzuki and Tsuchihashi (1970), based on which lip prints are categorized as follows:[5]

- Type I - A clear-cut groove running vertically across the lip
- Type I′ - Partial-length groove of type I
- Type II - A branched groove or a Y-shaped pattern
- Type III - An intersected groove or crisscross pattern
- Type IV - A reticular or checkered fencelike pattern
- Type V - Other or undetermined pattern.

Sex determination utilizing lip prints was pioneered by Vahanwala *et al*., (2000)[6,7] based on the numerical superiority of certain morphological patterns seen in either sex, in their study [Table 1]. These criteria were used in this study with the objective of assessing the sex of an individual. We have attempted to assess both cheiloscopy in the sex determination of an individual and the efficacy of lysochrome in developing invisible lip prints generated by the use of a persistent lipstick.

Materials and Methods

The current study was undertaken among 100 individuals (50 male and 50 female) selected from our institution by a simple randomized technique. Institutional ethical clearance was obtained prior to the study; the subjects were briefed regarding the procedure and purpose of the study, and lip prints were collected after obtaining written consent from all of them. Those with any known hypersensitivity to lipsticks and those with congenital or pathological abnormalities, inflammation, or trauma were excluded from the study.

Table 1: Criteria for sex determination, as suggested by Vahanwala *et al.*

Lip pattern	Region of occurrence	Predominantly seen in
Types I and I′	1st quadrant	Female
Type II	2nd quadrant	Male
Type III	Never occurs in lower lip	If occurring, only in male
Varied patterns	In all quadrants	Male
Same (alike) patterns	In all quadrants	Female

The materials used in the study were the persistent lipstick Revlon ColorStay Overtime® [ingredients: Isododecane, dimethicone, trimethylsiloxysilicate, polyethylene, disteardimonium hectorite, C12-15 alkyl benzoate, methicone, serica (silk powder), silica, propylene carbonate, butylhydroxytoluene (BHT), sorbic acid, caprylyl glycol, and 1,2-hexanediol]; for obtaining the latent lip prints, cellophane sheets; for developing the latent lip prints: Fingerprint-dusting feather brushes (round and flat), lysochrome dye Sudan Black B (C.I. 26150); for microscopy fat staining: (Rolex Chemical Industries, Mumbai, India); for digitizing the print and dividing it into quadrants: HP Deskjet F4283 all-in-one (scanner), Hewlett-Packard China, and Adobe® Photoshop® CS3 software, Adobe systems incorporated, USA.

Method of collection of the lip prints

The lips of each subject were cleaned thoroughly and lipstick was applied uniformly to the lips using lipstick applicator brushes, starting at the midline and moving laterally. The subject was then asked to rub his/her lips together to spread the lipstick uniformly. The lipstick was allowed to dry for about 2-3 min, based on manufacturer's specifications. After this, the lip print was obtained using cellophane paper, with the paper dabbing the center of the lips and then being pressed uniformly to the left and right corners of the lips. Care was taken to avoid sliding of the lips to prevent smudging of the print. The invisible lip print was then obtained. The lysochrome reagent Sudan Black B was dusted onto the lip print by using a dusting brush. It took 2 min for each print to develop. The print was then digitized by using a digital scanner, and coded by assigning it a definite number. All prints were evaluated by two different examiners, who were knowledgeable about the criteria for classification and sex determination [Figure 1].

Statistical analysis

All the data was analyzed using the SPSS statistical package version 17, IBM Corporation, USA to determine the frequencies and percentage of occurrence of the pattern types in each population group as well as a comparison between males and females, for which the data were subjected to the Chi-square test and the level of significance was kept at 0.05. The level of agreement between each examiner was also assessed for accuracy by determining the confidence interval (CI).

Results

The study was conducted on 100 healthy individuals (including 50 males and 50 females), with ages ranging

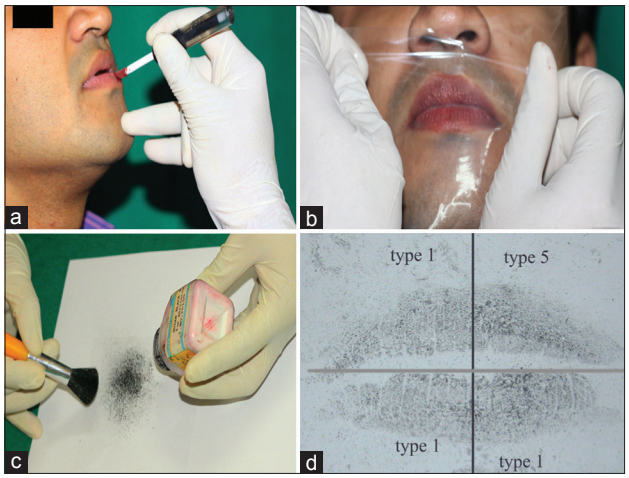

Figure 1: (a) Application of persistent lipstick with the applicator brush (b) Lifting of latent lip print with cellophane sheet (c) Application of lysochrome dye powder with round brush (d) Final lip print after digitization and division into quadrants

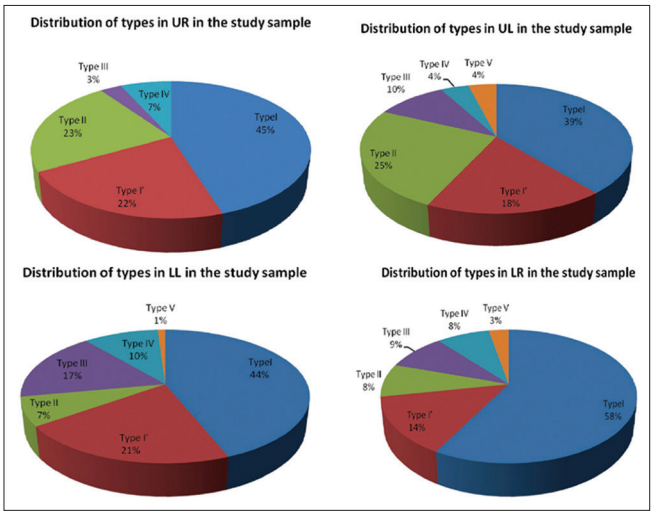

Figure 3: Pie diagram illustrating the distribution of samples in the UR, UL, LL, and LR quadrants

18-50 years, to assess cheiloscopy in sex determination from latent lip prints developed using lysochrome dyes. The developed latent lip prints exhibited all the variants described in the Suzuki and Tsuchihashi (1970)[5] classification [Figure 2].

In our study we found an association between gender and lip pattern to be statistically significant ($P < 0.001$) [Table 2]. In the female population, Type I (61%) was found to be more prevalent, followed by Type I′ (28%), Type II (9%), Type III (2%), Type IV (1%), and Type V (1%), while in the male population, Type I (33%) was found to be more prevalent, followed by Type II (23%), Type III (18%), Type IV (14%), Type I (10%), and Type 5 (3%) [Table 2], and these findings were in accordance with those of earlier researchers such as Vahanwala-Parekh (2000),[6,7] Sharma *et al*. (2000),[8] Saraswathi (2009),[9] Vats *et al*. (2012),[10] and Kautilya (2013).[11]

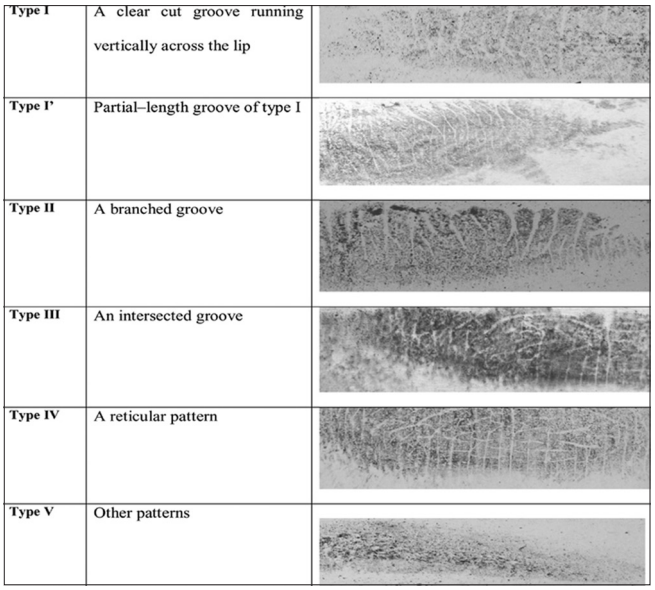

Figure 2: Variations in the lip prints obtained were classified using Suzuki and Tsuchihashi's classification

Table 2: Illustrates the frequency and repetition of different patterns in both genders

Type	Female		Male		Total	$\chi^{2\dagger}$	*P*
	n	%	*n*	%			
Type I	121	61	65	33	186	99.826	<0.001*
Type I′	55	28	20	10	75		
Type II	18	9	45	23	63		
Type III	3	2	36	18	39		
Type IV	1	1	28	14	29		
Type V	2	1	6	3	8		
Total	200	100	200	100	400		

*Denotes significant association; †Denotes Chi-square value

Table 3: Lip pattern type in UR quadrant according to gender

Type	Female		Male		Total	$\chi^{2\dagger}$	*P*
	n	%	*n*	%			
Type 1	31	62	14	28	45	20.126	<0.001*
Type 1′	12	24	10	20	22		
Type 2	7	14	16	32	23		
Type 3	0	0	3	6	3		
Type 4	0	0	7	14	7		
Total	50	100	50	100	100		

*Denotes significant association; †Denotes Chi-square value

Type I (45%) was the most commonly occurring lip print [Table 2]. In the upper right (UR) quadrant, it was found that Type I was more prevalent in females and Type II in males, and Type III and Type IV occurred only in males [Table 3 and Figure 3]. In the upper left quadrant (UL), it was found that Type I (39%) was the most prevalent lip pattern based on numerical superiority [Figure 2]. Type I and Type I′ were found to be more prevalent in females and Type II in males. Type III and Type IV were found

only in males [Table 4]. In the lower left quadrant (LL), the most prevalent lip pattern was Type I (44%), based on numerical superiority [Figure 3], and Type I and Type I' were found to be more prevalent in females; and Type III and Type IV more so in males. Type II was found to be present in almost equal frequency, and Type V was found only in one male [Table 5]. In the lower right (LR) quadrant, the most prevalent pattern based on numerical superiority was Type I (58%) [Figure 3]. Type I' was found to be more in females, Type III and Type IV only in males [Table 6].

The observer agreement of each examiner with the known standard was found to be very strong for males and females [Table 7].

The procedures of sex determination, if accurately done, have to be reproducible— there should not be an arbitrary decision if it is to be efficacious in personal identification.

Interobserver agreement between the two examiners was matched by assessing Cohen's kappa coefficient and the agreement of each examiner with standard [Table 7]. The agreement between the two examiners and the agreement of the examiners with the known gender were also assessed [Table 8]. Reliability of the estimate of accuracy between the examiners was assessed by finding the CI in each, which showed a high level of accuracy [Tables 7-9].

Discussion

This study was conducted to assess the efficacy of lysochrome in developing latent lip prints and to assess if the study of such lip prints could be of value in sex determination. The study subjects, selected randomly, were 100 healthy adult individuals from among the outpatients visiting the Department of Oral Medicine and Radiology, Dr. Syamala Reddy Dental College, Hospital and Research Centre, Bangalore.

The study conducted by Navarro *et al*. (2006) proved that Sudan Black B was the most efficacious dye for the development of latent lip prints from a human corpse's skin when compared to other lysochrome dyes such as Sudan III and Oil red O.[3] However, Navarro *et al*. (2007)[12] in a later study and other researchers including Kumar *et al*. (2010)[13] proved that fluorescent dyes could give results comparable with lysochrome dyes such as Sudan Black B. Singh *et al*. (2010),[14] conducted a similar study using Sudan Black B, on bone china cups, and satin and other cotton fabrics, from which they concluded that natural dyes such as vermilion and indigo gave results comparable to Sudan Black B in developing latent lip prints. In our study we have made use of the most efficacious lysochrome dye, Sudan black B [as per Navarro *et al*. (2006)].[3] We were able to develop the latent lip prints generated by the use of a persistent lipstick, and were able to classify the lip patterns thus revealed by applying the Suzuki and Tsuchihashi (1970) classification.[5] This is a "proof of concept" that lysochrome dyes are effective in developing latent lip prints generated by persistent lipsticks. However,

Table 4: Lip pattern type in UL quadrant according to gender

Type	Female		Male		Total	$\chi^{2\dagger}$	*P*
	n	%	*n*	%			
Type 1	30	60	9	18	39	51.637	<0.001*
Type 1'	16	32	2	4	18		
Type 2	3	6	22	44	25		
Type 3	0	0	10	20	10		
Type 4	0	0	4	8	4		
Type 5	1	2	3	6	4		
Total	50	100	50	100	100		

*Denotes significant association; †Denotes Chi-square value

Table 5: Lip pattern type in LL quadrant according to gender

Type	Female		Male		Total	$\chi^{2\dagger}$	*P*
	n	%	*n*	%			
Type 1	31	62	13	26	44	34.814	<0.001*
Type 1'	14	28	7	14	21		
Type 2	3	6	4	8	7		
Type 3	1	2	16	32	17		
Type 4	1	2	9	18	10		
Type 5	0	0	1	2	1		
Total	50	100	50	100	100		

*Denotes significant association; †Denotes Chi-square value

Table 6: Lip pattern type in LR quadrant according to gender

Type	Female		Male		Total	$\chi^{2\dagger}$	*P*
	n	%	*n*	%			
Type 1	29	58	29	58	58	21.897	0.001*
Type 1'	13	26	1	2	14		
Type 2	5	10	3	6	8		
Type 3	2	4	7	14	9		
Type 4	0	0	8	16	8		
Type 5	1	2	2	4	3		
Total	50	100	50	100	100		

*Denotes significant association; †Denotes Chi-square value

Table 7: Agreement of each examiner with standard (known gender)

Examiner	#Inspected	#Matched	% Matched	95% CI
Examiner 1	100	94	94	87.40-97.77
Examiner 2	100	93	93	86.11-97.14

Examiner	Response	Kappa (κ)	SE (κ)	*Z*	*P*
Examiner 1	F	0.880	0.100	8.800	<0.001*
	M	0.880	0.100	8.800	<0.001*
Examiner 2	F	0.860	0.100	8.600	<0.001*
	M	0.860	0.100	8.600	<0.001*

*Denotes significant agreement, The level of agreement of Examiner 1 with the standard (known gender) was found be very strong for both males (κ=0.880) and females (κ=0.880), The level of agreement of Examiner 2 with the standard (known gender) was found be very strong for both males (κ=0.860) and females (κ=0.860), CI: Confidence interval, SE: Standard error, #: Subjects

Table 8: Agreement between the two examiners

#Inspected	#Matched	% Matched		95% CI
100	97	97		91.48-99.38
Response	**Kappa (κ)**	**SE (κ)**	***Z***	***P***
F	0.940	0.100	9.399	<0.001*
M	0.940	0.100	9.399	<0.001*

*Denotes significant agreement, The level of agreement of between the two examiners was found be very strong for both males (κ=0.940) and females (κ=0.940), CI: Confidence interval, SE: Standard deviation, #: Subjects

Table 9: Agreement of both examiners with standard (known gender)

#Inspected	#Matched	% Matched		95% CI
100	92	92		84.84-96.48
Response	**Kappa (κ)**	**SE (κ)**	***Z***	***P***
F	0.870	0.071	12.303	<0.001*
M	0.870	0.071	12.303	<0.001*

*Denotes significant agreement, The level of agreement between the two examiners and the standard (known gender) was found be very strong for both males (κ=0.870) and females (κ=0.870), CI: Confidence interval, SE: Standard error, #: Subjects

it is prudent to keep in mind that the application of lysochrome powder must be done using fine dusting brushes specially designed for forensic examinations, mainly so as not to smudge the print or allow "clumping" of the powder in specific areas.

The results obtained from our study were not in accordance with those from studies conducted by Augustine *et al*. (2008),[15] Gondivkar *et al*. (2009),[16] Patel *et al*. (2010),[17] Wadhwan *et al*. (2011),[18] Singh *et al*. (2011),[19] and Malik *et al*. (2011),[20] which could be attributed to the variable sample size of subjects used by different researchers, the use of traditional lipsticks, operator dependency, and also the presence of prominent facial hair in men that could have led to smudging of the print. However, irrespective of the findings from the studies conducted, all the researchers have opined positively about the uniqueness of lip prints and their application in sex determination and in establishing the identity of an individual. Contrary to the recent studies, which have all followed the Sivapathasundaram *et al*.[21] recommendation of utilizing the lower front portion of the lips as a prominent region of interest because it is almost always present on any lip print obtained, we adhered to the original method of Vahanwala *et al*. (2000).[6,7] According to the latter method, we divided the lip prints we obtained into four quadrants, and analyzed the lip patterns in each quadrant. This revealed statistically significant results, and among them, no two lip prints were identical as far as the arrangement of lip patterns was concerned, thus proving the uniqueness of each lip print— in accordance with the findings by Suzuki *et al*. (1970).[5]

In our study, we found that Type III (2%), Type IV (1%), and Type V (1%) [Table 3] were found in the female population, which was not in accordance with the criteria as per Vahanwala-Parekh (2000),[6,7] which probably caused errors in sex determination. But these can be ruled out as minor variations in the population; they may reveal a different scenario if the study is performed on a larger sample size.

Conclusions

From our study it was concluded that cheiloscopy holds potential for use in sex determination. The current research on latent lip prints has shown a considerably new aspect of cheiloscopy, and the use of lysochrome dyes in our study has proved its effectiveness as an agent to develop latent lip prints. The use of such dyes could pave the way for future research on the development of latent lip prints and their application in sex determination. Although our study was able to achieve a fair degree of accuracy in sex determination, often, in a legal scenario, the difference between positive and negative identification means the difference between the life and death of an individual. Hence, the results obtained in our study do not prove ours to be a foolproof method of sex determination; nevertheless it takes us one step closer to the truth. Earlier studies had not explored the option of setting up a database of lip prints, but the software analysis employed in our study allowed us to easily classify and maintain a database of lip prints for further perusal. We are aware that the advanced methods of developing lip prints including latent lip prints at a crime scene are still confined to laboratories and research institutions.[22] The only possible way to overcome this and bring cheiloscopy into day-to-day practice is to introduce it as a procedure of significance in criminalistics and the syllabi of training in forensic odontology. The classification and observation of lip patterns in an Indian population has resulted in useful data, and there has been progress in research focused on sex determination. It will open up newer avenues that could contribute extensively toward criminal investigation and personal identification. Thus, cheiloscopy holds potential as a tool for sex determination of an individual, among other modalities, to identify an individual. Further, studies conducted using the same methods in our country in a larger population involving more subjects are recommended so that it can be assessed whether demonstrable gender difference exists that is identifiable with cheiloscopy.

This study proved that lysochrome dyes are highly effective in the development of latent lip prints and that lip prints thus obtained can be used for the sex determination of an individual with fair accuracy. If further research is not focused in the realm of latent lip prints, there is a serious risk of the slow death of lip print analysis as a modality of forensic investigation.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have

given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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