ISSN 0973-2063 (online) 0973-8894 (print)

Bioinformation 22(10): 7046-7049 (2026)

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BIOINFORMATION
Discovery at the interface of physical and biological sciences

BIOMEDICAL INFORMATICS

www.bioinformation.net

Volume 22(10)

Research Article

Received October 1, 2026; Revised October 31, 2026; Accepted October 31, 2026, Published October 31, 2026

DOI: 10.6026/973206300227046

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478
2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

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Citation: Solanke *et al*. Bioinformation 22(10): 7046-7049 (2026)

Comparative evaluation of cheiloscopy and mandibular canine index for gender determination: A cross-sectional study

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ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(10): 7046-7049 (2026)
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Abstract:
Determination of sex is a fundamental step in forensic identification. Therefore, it is of interest to evaluate and compare the cheiloscopy and mandibular canine index (MCI) in 100 adults (50 males, 50 females) aged 18–30 years. Lip-print patterns showed no significant sex differences ($p > 0.05$), although vermilion powder produced clearer prints than Sudan black II (62% vs. 18%; $p < 0.001$), with an overall cheiloscopy accuracy of 55%. Mesiodistal canine widths, inter-canine distance and MCI were significantly greater in males ($p < 0.01$) and MCI correctly classified 85% of subjects. The mandibular canine index was more accurate than cheiloscopy for sex determination, while lip-print analysis may remain useful when dental evidence is unavailable.

Keywords: Cheiloscopy, lip prints (LPs), mandibular canine index, sexual dimorphism, gender determination, forensic odontology, vermilion, Sudan black

Background:
Establishing personal identity is fundamental to legal and humanitarian investigations, with sex determination serving as an important initial step in forensic identification **[1].** When conventional methods such as fingerprints, DNA, or visual recognition are unavailable because of mass disasters, decomposition, or fragmented remains, alternative identification methods become important **[2]**. Cheiloscopy is the study of grooves and furrows on the vermilion border of the lips, which show individual variation and relative stability throughout life **[3]**. Lip print classification was systematized by Suzuki and Tsuchihashi into six major pattern types, providing a standardized approach for forensic analysis **[4]**. Lip prints may be deposited as latent traces on objects such as cups, glasses and cigarette ends and various dyes have been investigated for their development and visualization **[5]**. Teeth are among the most durable structures in the human body and can resist putrefaction, fire and trauma, making them valuable for medico-legal identification **[6]**. The mandibular canine exhibits considerable sexual dimorphism and has therefore been investigated as a useful dental marker for sex determination **[7]**. Therefore, it is of interest to compare and evaluate the cheiloscopy and MCI within the same cohort may help determine their relative accuracy for sex identification.

Materials and Methods:
This cross-sectional study was conducted after institutional ethics approval and written informed consent from all participants. Fifty male and fifty female subjects (aged 18–30 years) were selected from college students and the outpatient department. For cheiloscopy, subjects had lips free of any pathology; subjects with lesions, inflammation, trauma, deformity, surgical scars are known sensitivity to lipstick was excluded. For the mandibular canine index, only subjects with Angle's Class I molar and canine relationship, healthy gingiva and periodontium, caries free teeth and normal overjet and overbite were included; those with malocclusion, carious, attrited or malposed canines, or spacing or crowding in the mandibular anteriors were excluded. Evaluation of latent lip prints: long-lasting lipstick was applied uniformly to the subject's lower lip and left for five minutes. The subject held a drinking glass in lip contact for five seconds and two latent lower-lip prints were obtained. One print was developed with natural dye (vermilion powder) and the other with lysochrome dye (Sudan black II) until the print was reproduced, then transferred to white paper with transparent cellophane tape. The middle portion of each print was examined under a magnifying lens and classified by the Suzuki and Tsuchihashi system (Type I, complete vertical; Type I, partial vertical; Type II, branched; Type III, intersected; Type IV, reticular; Type V, undifferentiated). Development quality was graded as Good (outline and grooves easily studied), Fair (outline visible, grooves less clear), or Poor (outline visible but grooves not appreciable). Evaluation of the mandibular canine index: alginate impressions of both arches were made and casts poured in dental stone. The mesiodistal width of the right and left mandibular canines (greatest width between contact points) and the inter canine distance (between canine tips) were measured on the cast with a digital vernier caliper. The observed MCI was calculated as the mesiodistal canine width divided by the mandibular inter-canine width. A standard MCI cut off was derived as ((mean male MCI − SD) + (mean female MCI + SD)) / 2, values at or below the cut-off being classified female and those above as male. Lip-print patterns and canine measurements were analysed using the chi-square test and the Mann–Whitney U test; canine parameters were compared with the independent samples t-test. The accuracy of each method was assessed by discriminant function analysis, with significance set at $p < 0.05$.

Results:

Latent lip prints were reproduced in 100 subjects (mean age 22 years). With natural dye (vermilion), Type I was the most common pattern in both sexes and Type V the least common; the distribution did not differ significantly between males and females (chi-square = 4.46, p = 0.485; Mann–Whitney U test p = 0.705) **(Table 1).** With lysochrome dye (Sudan black II), Type I again predominated in both sexes and the distribution was likewise not significant (chi-square = 4.52, p = 0.478; Mann–Whitney U p = 0.495) **(Table 2).** Clarity of development differed markedly between the two reagents: vermilion powder produced "good" prints in 62% of cases versus only 18% with Sudan black II, a statistically significant difference (chi-square = 35.07, p < 0.001), indicating superior and more reproducible development with the natural dye. On discriminant function analysis, cheiloscopy correctly classified 66% of males and 44% of females, for an overall accuracy of 55%. For the mandibular canine index, all measured parameters were significantly greater in males than females **(Table 3).** The mesiodistal width of the right mandibular canine was 6.99 ± 0.26 mm in males versus 6.50 ± 0.47 mm in females and of the left canine 6.97 ± 0.23 mm versus 6.45 ± 0.44 mm (both p < 0.001). Right and left MCI were 0.27 ± 0.01 in males versus 0.26 ± 0.01 in females (both p < 0.001) and inter-canine distance was 26.07 ± 1.60 mm versus 25.21 ± 1.53 mm (p = 0.007). The derived standard MCI cut-off was 0.265. Using this cut-off, 64% of males and 52% of females were correctly identified (overall accuracy 58%), whereas discriminant function analysis using all canine parameters raised accuracy to 96% of males and 74% of females, for an overall accuracy of 85% **(Table 4).**

Table 1: Distribution of lip-print patterns by sex using natural dye (vermilion powder)

Lip-print type (natural dye)	Male, n	%	Female, n	%	Total
Type I	17	34.0	18	36.0	35
Type I′	7	14.0	11	22.0	18
Type II	10	20.0	5	10.0	15
Type III	9	18.0	8	16.0	17
Type IV	4	8.0	7	14.0	11
Type V	3	6.0	1	2.0	4
Total	**50**	**100**	**50**	**100**	**100**

Chi-square = 4.46, p = 0.485 (not significant)

Table 2: Distribution of lip-print patterns by sex using lysochrome dye (Sudan black II)

Lip-print type (Sudan black II)	Male, n	%	Female, n	%	Total
Type I	21	42.0	18	36.0	39
Type I′	6	12.0	10	20.0	16
Type II	9	18.0	4	8.0	13
Type III	6	12.0	6	12.0	12
Type IV	6	12.0	7	14.0	13
Type V	2	4.0	5	10.0	7
Total	**50**	**100**	**50**	**100**	**100**

Chi-square = 4.52, p = 0.478 (not significant)

Table 3: Comparison of mandibular canine parameters between sexes (independent-samples t-test)

Parameter	Male (mean ± SD)	Female (mean ± SD)	P value
MD width, right canine (mm)	6.99 ± 0.26	6.50 ± 0.47	< 0.001
MD width, left canine (mm)	6.97 ± 0.23	6.45 ± 0.44	< 0.001
MCI, right canine	0.27 ± 0.01	0.26 ± 0.01	< 0.001
MCI, left canine	0.27 ± 0.01	0.26 ± 0.01	< 0.001
Inter-canine distance (mm)	26.07 ± 1.60	25.21 ± 1.53	0.007

MD, mesiodistal; MCI, mandibular canine index

Table 4: Accuracy of sex determination by cheiloscopy and the mandibular canine index

Method	Males correct (%)	Females correct (%)	Overall (%)
Cheiloscopy (discriminant analysis)	66	44	55
MCI, standard cut-off (0.265)	64	52	58
MCI (discriminant analysis)	96	74	85

MCI, mandibular canine index

Discussion:

Lip prints are identifiable from the sixth week of intrauterine life and their pattern rarely changes thereafter, giving them the persistence required of an identification tool and their development does not require lipstick because labial secretions permit latent prints **[7]**. In this study, Type I (complete vertical) was the predominant pattern in both sexes with each dye and the pattern distribution did not differ significantly between males and females consistent with reports by Tsuchihashi, Vahanwala and Parekh and others who found Type I common in females, though several investigators have instead reported intersected or reticular patterns as most frequent, underlining the population variability of lip print morphology **[8]**. A practical finding was that vermilion powder developed latent prints significantly more clearly than Sudan black II (62% vs 18% "good"), agreeing with Singh *et al.*, who reported natural dyes to be comparable or superior to lysochromes; vermilion is also inexpensive and readily available and may serve as a substitute lysochrome for latent lip-print development **[9]**. Nevertheless, because the lip is a mobile structure, the same person can produce different prints depending on pressure, direction and the amount of lipstick and smudging and manual overlay introduce subjectivity limitations that constrain the reliability of cheiloscopy for sex determination, for which overall accuracy here was only 55%. In contrast, mesiodistal canine width, MCI and inter-canine distance were all significantly greater in males, in agreement with Gandhi *et al.* **[10]**. The greater sexual dimorphism of the canine is attributed to Y-chromosomal influence on tooth size that is not uniform across the dentition, whereas X-linked influence is more uniform **[11]**. The standard MCI cut off yielded modest accuracy (58%), consistent with authors such as Acharya *et al.* **[12],** who noted that the index a ratio of two absolute measurements may not capture the dimorphism present in the raw widths **[13]**. However, discriminant function analysis using the absolute canine measurements raised accuracy to 85%, substantially exceeding cheiloscopy and supporting the mandibular canine as the more dependable of the two methods **[14, 15]**.

Conclusion:

We show that lip print patterns did not differ significantly between sexes and gave an overall cheiloscopy accuracy of 55%, whereas mandibular canine measurements showed significant sexual dimorphism and reached 85% accuracy on discriminant analysis. The mandibular canine index is therefore the more accurate of the two methods for gender determination, while

cheiloscopy retains value as a supplementary aid particularly where dental structures are unavailable. Vermilion powder proved a clearer, cheaper and more accessible reagent than Sudan black II for developing latent lip prints.

Limitations:
This was a single center study of a narrow age band (18–30 years) chosen to minimise attrition and the sample was modest (n = 100), which may limit generalisability to other populations and age groups. Lip prints were recorded with lipstick and manual overlay, introducing potential smudging and observer subjectivity and only the middle third of the lower lip was classified. The standard MCI cut off, being a relative value, under performed compared with discriminant analysis of absolute measurements. Larger, multi center, population specific studies with standardised latent-print techniques are needed before either method is applied forensically.

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