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Preliminary study on morphometric analysis of the human scalp hair for discrimination of ethnic Malay and ethnic Chinese in Malaysia

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Abstract

Background: Hair is one of the most frequently encountered trace evidence in crime scenes. It has been used for differentiating races or identifying species of animals. An understanding of intra- and inter-variation of human scalp hair may enable discrimination based on ethnicity. This study aims to discriminate the ethnic Malays and ethnic Chinese in Malaysia based on three morphometric measurements of the human scalp hair.

Method: The sample group was composed of 100 Malaysians, 50 each (i.e. 25 males and 25 females) from ethnic Malays and ethnic Chinese. Each subject has provided 20 strands of hair. The hair width (HW), scale layer difference (SLD), and medullary index (MI) of each hair were determined using a compound microscope. Following that, the inter-variation of hair was evaluated according to (a) head regions, (b) sex, and (c) ethnic groups and assessed using descriptive and inferential statistics, i.e. independent *t* test and Wilcoxon's rank-sum test.

Results: Results show that Malays and Chinese are different in all the three morphometries. However, Malay males are different from females only in terms of SLD, and Chinese males and females could be discriminated using MI. Next, hair originating from upper and lower head regions, regardless of ethnicity and sex, are not significantly different.

Conclusion: In conclusion, Malays and Chinese could be discriminated based on morphometric measurements of scalp hair. The finding sheds new light on the feasibility of differentiating ethnic groups using physical measurements alone.

Keywords: Forensic sciences, Hair, Inter-variation, Intra-variation

Background

Hair is defined as a slender, thread-like outgrowth from a follicle in the skin of mammals. In brief, it is a dead cell which is mainly filled with keratin protein (Deedrick and Koch 2004). Hair can be physically divided into three parts, i.e. medulla, cortex, and cuticle (Robertson 1999), as illustrated by Fig. 1. The cuticle is the outermost layer of hair and served as the protective layer of hair. On the other hand, the cortex is referred to as the layer that lays in between the medulla and cuticle. This layer is composed of elongated, fusiform, and keratinized filaments aligned parallel to the length of the hair. The core of the hair is known as the medulla. It is a cellular column running through the center of the cortex.

The uniqueness of hair samples is attributed by the presence of keratin protein which provides a strong structural basic. The disulphide bonding in the keratin protein makes the hair resistant to various physical and chemical degradations (Kaszynski 1985). Therefore, hair can retain its characteristics for a long time without obvious physical deterioration. In addition, every individual loses about 100 strands of hair daily without being aware of it. The number of lost hair can be varied and affected by the health conditions as well as genetic of the person. Eventually, this explains why hair appears to be one of

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the most frequently encountered trace evidence in a crime scene.

Hair samples can be analysed in both forensic DNA and physical evidence laboratory. Due to the unique structural characteristics of hair, it has been claimed to be a good source for extracting mitochondrial DNA. Nonetheless, the cost for such DNA analysis can be very high and it requires sophisticated procedures like DNA sequencing. Furthermore, the mitochondrial DNA profile is not unique to each individual. Theoretically, mitochondrial DNA is maternally inherited, and thus, it is good for tracing the origin of a human but cannot be used to identify a particular person (Butler 2011; Takayanagi et al. 2003; Wilson et al. 1995a, 1995b). In a physical evidence laboratory, hair samples are examined according to physical properties that can be qualitative or quantitative in nature (Robertson 1999). The scale patterns, pigment distribution, and medulla types are examples of qualitative characteristics of hair. On the other hand, those measurable characteristics (i.e. quantitative) are collectively known as the morphometry of hair, including medullary index (MI), hair width (HW), and scale layer difference (SLD) (McCrone 1977). It has been known that animals and humans show different hair morphology and morphometric measurements. For instances, animals like cat, dog, and deer show distinct medullary patterns (Bisbing 1985).

Previous studies have confirmed that human hair of different ancestry, i.e. Negroid, Mongoloid, and Caucasian, are distinct (Bisbing 2002; Robertson 1999). However, reports on the differences in hair characteristics among different ethnic groups of a particular country are still very limited in the literature. Such insights can be useful in narrowing suspect according to ethnicity. Malaysia composes of three major ethnic groups, i.e. Malays, Chinese, and Indians, and several minority ethnic groups from Borneo. The aim of this work is to assess differences between Malays and Chinese in Malaysia according to MI, HW, and SLD. Statistical hypothesis testing is used to assess the variation of hair at a significance level of 0.05.

Methodology

Participants

The present study was conducted from June 2016 to June 2017 at the Universiti Kebangsaan Malaysia (UKM) campus at Bangi, Selangor, Malaysia. Ethical clearance for this study was obtained from the Jawatankuasa Etika Penyelidikan UKM. All subjects were informed about the purpose, nature, and possible risks of the study before written informed consent was obtained. The subjects were selected conveniently (i.e. convenience sampling method) and mainly undergraduate students from different faculties in UKM. A total of 100 Malaysian, 50 from each ethnic group (i.e. Chinese and Malays), were recruited in the study. In order to eliminate differences arisen from interracial marriage, only subjects who have confessed to have at least three generations of linear relatives were included in this study. In addition, subjects who have had hair treatment or dyeing over the last 3 months were also excluded (Seta et al. 1988; Nishikawa et al. 1998; Kuzuhara 2013). Table 1 shows the origins of the hair samples by head regions, sex, and ethnic group.

Data collection

Each subject was required to provide 10 strands of hair from the upper head region and another 10 strands of hair from the respective lower head region. The required number of hair was cut with scissors and kept in a labelled paper envelope. A microscope with an attached camera was used to measure hair width (HW), scale layer differences (SLD), and medulla index (MI) (Fig. 1). The compound microscope (Leica ICC50 HD, Switzerland) is equipped with the Leica Application Suite (LAS EZ) version 2.1.0 software. Figures 2 and 3 illustrate how to determine HW and MI and an example of ten readings of SLD, respectively. The SLD is the average value of ten distances between two adjacent scales on the same hair, whilst MI is computed as follows (Eq. 1):

$$\mathrm{MI} = \frac{\text{Medulla width}}{\text{Hair width}} \quad (1)$$

Data analysis

The differences between ethnicity, sex, and head regions were first assessed using descriptive statistics. Mean ($\overline{x}$) and standard deviation (SD) values are estimated as follows:

$$\overline{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \quad (2)$$

$$\mathrm{SD} = \sqrt{\frac{\sum_{i=1}^{n}(x_i-\overline{x})^2}{n-1}} \quad (3)$$

Table 1 Origins of the 2000 hair samples

Ethnic group	Malays		Chinese	
Sex	Male	Female	Male	Female
Number of subjects (*n*)	25	25	25	25
Head region				
Upper	250	250	250	250
Lower	250	250	250	250
Total	500	500	500	500

Fig. 1 Cross-section of human scalp hair observed under compound light microscope. Photo credit to Lee Yong Ming

where x refers to HW, MI, or SLD and n denotes the total number of readings of the studied parameter. In order to gain more insights, the parameters are also explored using two-tailed hypothesis tests and the respective null hypotheses are expressed as follows:

$$H_0 : \overline{x}_{\text{lower head region}} = \overline{x}_{\text{upper head region}} \quad (4)$$

$$H_0 : \overline{x}_{\text{male}} = \overline{x}_{\text{female}} \quad (5)$$

$$H_0 : \overline{x}_{\text{Malays}} = \overline{x}_{\text{Chinese}} \quad (6)$$

Prior to hypothesis test, the Kolmogorov-Smirnov test and Levene's test for equality of variances were implemented to assess the assumption of data normality and equal variances, respectively. Results showed that not all the parameters were normally distributed or/and showed equal variances. For that reason, both the unpaired t test and Wilcoxon's rank-sum test (Chen 2003) have been conducted. One can say the difference is significant if both the tests present p value less than 0.05. All the statistical tests were conducted using the R software (version 3.3.2) (R Development Core R Team 2016).

Results

Descriptive statistics

Table 2 shows the descriptive statistics of three different morphometries of hair according to (a) ethnic group, (b) sex, and (c) head region. Overall, hair originating from upper and lower head regions show similar mean values. This seems to indicate that hair collected from different head regions of an individual tend to be similar. However, Chinese males and females exhibited different hair width that males showed coarse hair shaft than females. However, a similar observation cannot be seen in Malay

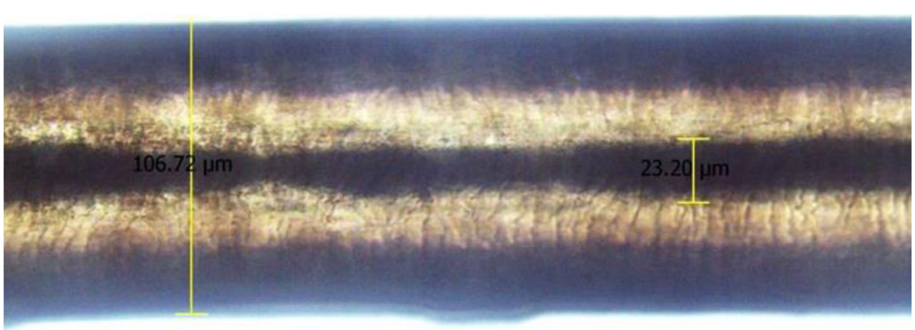

Fig. 2 Measurement of hair width (HW) and medullary width (MW)

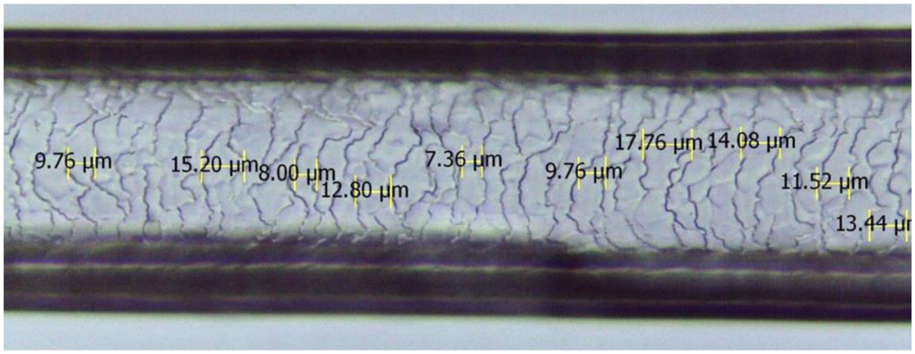

Fig. 3 Scale layer difference (SLD) is the mean value of ten measurements on a single strand of hair

males and females. The differences in the morphometry of hair were much obvious when examined by ethnic group. Chinese tended to show a wider hair width than Malays, regardless of the sex and head regions. On the other hand, the hair of Malay origin showed higher SLD values than the hair of Chinese origin. The MI values of Malay hair were lower than that of Chinese hair.

In brief, both ethnic groups showed obvious differences in all the three studied morphometries. However, Malay males and females can only be discriminated according to SLD, whereas Chinese males and females showed different HW values.

Hypothesis test

The same data used in calculating the descriptive statistics were further assessed using a hypothesis test. The p values estimated via t test and Wilcoxon's rank-sum test are presented in Table 3. It is important to note that the difference between head regions was assessed using the paired t test, and inter-sex and inter-ethnic variation were evaluated using the unpaired t test.

The intra-person variation was assessed according to inter-head regions differences. For that, the 2000 samples were divided into eight groups, i.e. upper region and lower region, by sex and ethnicity. The purpose of hypothesis tests was to assess if the hair collected from the upper region are significantly different from those obtained from the respective lower head region. Overall, it is clearly seen that the hair of either Malay or Chinese origin were quite homogeneous, because both the parametric and non-parametric tests produce similar outcomes, i.e. p value < 0.05. However, Chinese males showed inhomogeneous MI and Malay females showed different SLD values.

Next, it is noted that males and females from Malay and Chinese sub-populations, respectively, could be differentiated using SLD and MI values because the associated p values are highly significant, i.e. p value < 0.001. Contrarily, inter-sex variation as demonstrated using HW for both ethnic groups is of no forensic values. This is especially true for Malay subjects since the p value is approaching 1.00.

Following that, the inter-ethnic difference in morphometry of hair was examined carefully. Based on Table 3c, all three parameters could be used to differentiate the two ethnic groups because the p values are far less than 0.001. By referring to the absolute p values, the three morphometries of hair could be ranked according to the reliability in differentiating Malays and Chinese in descending order: SLD > MI > HW.

Table 2 Summary statistics of the morphometry of hair by ethnicity, sex, and head regions

Ethnicity	Sex	Head region	Mean ± SD		
(n = 1000)	(n = 500)	(n = 250)	HW (um)	SLD (um)	MI
Malays	Male	Upper	81.90 ± 18.82	8.22 ± 0.96	0.19 ± 0.08
		Lower	80.65 ± 23.47	8.22 ± 1.05	0.18 ± 0.08
	Female	Upper	81.94 ± 16.32	8.45 ± 1.08	0.19 ± 0.07
		Lower	80.70 ± 16.38	8.63 ± 1.05	0.19 ± 0.07
Chinese	Male	Upper	87.30 ± 19.50	7.54 ± 0.83	0.23 ± 0.09
		Lower	87.02 ± 17.84	7.66 ± 0.78	0.21 ± 0.09
	Female	Upper	86.03 ± 16.63	7.52 ± 0.66	0.23 ± 0.09
		Lower	86.53 ± 15.95	7.55 ± 0.68	0.24 ± 0.09

HW hair width, *SLD* scale layer difference, *MI* medullary index, *n* sample size

Table 3 Statistical test results of *t* test (*t*) and Wilcoxon's rank sum test (W)

(a) Inter-head regions						
Parameter	Test	Malays		Chinese		
		Male	Female	Male	Female	
HW	*t*	0.51	0.40	0.87	0.73	
	W	0.44	0.33	0.84	0.79	
SLD	*t*	0.94	0.05*	0.31	0.62	
	W	0.89	0.04*	0.24	0.61	
MI	*t*	0.13	0.81	0.01*	0.36	
	W	0.22	0.96	0.01*	0.33	
(b) Inter-sex						
Ethnicity	HW		SLD		MI	
	t	W	*t*	W	*t*	W
Malays	0.97	0.84	< 0.001*	< 0.001*	0.07	0.34
Chinese	0.43	0.55	0.40	0.53	< 0.001*	< 0.001*
(c) Inter-ethnic						
Test	HW		SLD		MI	
t test	3.85×10^{-11}*		1.27×10^{-84}*		1.71×10^{-25}*	
Wilcoxon's test	9.16×10^{-12}*		1.43×10^{-75}*		1.38×10^{-16}*	

HW hair width, *SLD* scale layer difference, *MI* medullary index
**p* value less than or equal to 0.05

Discussion

To the best of our knowledge, there is only one work which attempted to study hair morphology in a Malaysian population (Nataraja and Jessica 2015). In that work, the authors have assessed differences between the three major ethnic groups in Malaysia, i.e. Chinese, Malays, and Indians, using only qualitative features of hair, i.e. medulla patterns, cuticle thickness, and inner cuticle margin. In addition, the observed differences were assessed only with descriptive statistics. As a result, this work is the first report that studied the inter-ethnic difference in Malaysian sub-population using quantitative characteristic of hair, and both descriptive and inferential statistics were employed to evaluate the difference. Basically, the findings are in line with that presented by Nataraja and Jessica (2015), i.e. Malays and Chinese could be discriminated based on hair morphology.

On the other hand, some studies concentrated on the discriminatory power of hair morphology in populations other than Malaysian. For instance, it has been reported that twins could be discriminated by using common microscopic hair characteristics (Das-Chaudhuri 1976). However, Sharma et al. (2002) reported contradicting findings when involving more number of twins in a similar study. However, both works focused only on twin subjects. In contrary, this study has collected subjects according to ethnicity and involved no twins.

However, the results presented here is contradicted to other similar works involving non-Malaysian subjects (Kaur and Kumar 2000; Jasuja and Minakshi 2002; Aitken and Robertson 1986; Aitken and Robertson 1987). Those works concluded that the hair morphological parameters were not suitable for forensic casework because the variation was not consistent across two different populations (Kaur and Kumar 2000) or within one specific ethnic group (Jasuja and Minakshi 2002) or even for a particular individual (Aitken and Robertson 1986; Aitken and Robertson 1987; Robertson 1982).

Kaur and Kumar (2000) have considered the ethnic differences between Brahmin and Rajput populations in India. Theoretically, the genotype-specific difference between Malays and Chinese in Malaysia would be higher than that presented by the Brahmin and Rajput populations. This is due to the fact that Malaysian Chinese ancestors are those who migrated from Mainland China upon British colonization. On the other hand, Brahmin and Rajputs are believed to be closely related to each other (Mohan 2016). In fact, this is in line with the fact that hair morphology is partly controlled by genetic factors (Shimomura and Christiano 2010).

On the other hand, Jasuja and Minakshi (2002) have drawn a conclusion based solely on descriptive statistics. They studied intra-variation of hair within one specific ethnic group. However, they have not considered inferential statistics but only descriptive statistics in deriving the conclusion. In contrary, this work employed both the descriptive and inferential statistics to evaluate differences in hair morphology. As such, it seems relevant to suggest that the hypothesis test shall be conducted to confirm observations derived from the descriptive statistics.

Recently, Houck and Budowle (2002) have demonstrated that microscopic analysis of hair is as useful as molecular analysis because both approaches relied on independent types of information. For that reason, it seems worth to invest more effort to explore and assess the discriminatory power of other morphometry of hair in identifying a particular ethnic group (Apama and Yadav 2013). Following that, the study can be expanded later by considering both hair morphology and morphometry properties concurrently to achieve more insights on the subject of concern. In addition, an attempt should be made to construct a prediction model using multivariate modelling algorithm such as linear discriminant analysis or partial least squares-discriminant analysis.

The primary limitation of this work is the small sample sizes. In the context of statistical analysis, sample sizes could affect the reliability of hypothesis test. Due to that reason, we have derived the conclusion with regard to statistical differences by referring to the parametric (i.e. *t* test) as well as non-parametric (i.e. Wilcoxon's rank-sum test) statistics. Based on Table 3, none of the pair of hypothesis tests shows disagreement in terms of statistical significance. As such, we could say that the impact of small sample sizes is minor in this work.

Conclusion

In this study, differences between two ethnic groups in Malaysia, i.e. Malays and Chinese, have been assessed using the morphometry of hair. In conclusion, this study revealed that Malays and Chinese could be differentiated according to hair width, medullary index, and scale layer differences. The inter-variations are found to be statistically significant. Physical analysis of human hair is a cost-effective procedure in comparison to advanced molecular biology techniques. Hence, this study has provided vital insights on the feasibility of differentiating ethnic groups in Malaysia based solely on physical hair measurements.

Abbreviations

DNA: Deoxyribonucleic acid; HW: Hair width; MI: Medullary index; SLD: Scale layer difference; UKM: Universiti Kebangsaan Malaysia

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Authors' contributions

SSA and KLO performed the volunteer hair collection, data collection, assembly, and analysis. LCL performed the statistical analysis and drafted the manuscript. WNS conceived the experiment and initial experimental design. All authors read and approved the final manuscript. SSA and KLO were undergraduate students registered under the program of forensic science at UKM. The article was prepared based on their final year projects. Both of them were supervised by WNS and LCL.

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Availability of data and materials

All relevant data are within the paper.

Ethics approval and consent to participate

This study was performed after obtaining clearance from the Jawatankuasa Etika Penyelidikan UKM and written informed consent from the participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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