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Original Article

The Role of Lipsticks and Blush Sticks in Genetic Profiling for Human Identification

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Abstract

The core objectives of the current study are to generate human DNA profiles from used lipsticks and blush sticks of various brands available in Pakistan. A total of 12 international and local brands of lipsticks and blush sticks were selected. The lipsticks and blush sticks were applied by twenty different healthy female volunteers of 21-30 years of age. The heads of used lipsticks and blush sticks were swabbed with dry sterile cotton swabs. The qualitative and quantitative analysis was done by real time polymerase chain reaction (PCR), using a Quantifiler® Duo DNA Quantification Kit on Real Time PCR ABI™ 7500. Samples were amplified for 16 STR loci using an AmpFlSTR® Identifiler® PCR amplification kit on Thermocycler ABI 9700. The amplified product was run on Applied Biosystems 3130™ Genetic Analyzer. The genetic profiles were analyzed on GeneMapper® ID-X software version 1.0. The quantification results showed that the yield of DNA obtained from lipstick samples was greater than that of DNA obtained from blush stick samples. The real-time PCR results showed that only 16% of cosmetic samples had shown inhibition. The DNA profiles obtained from all blush stick samples were of good quality compared to those from lipstick samples. No profile was obtained from one blush stick sample (DNA 0.001 ng/μL) and four lipstick samples (DNA 0.001-0.003 ng/μL) because the amount of DNA in each of these samples was less than the amount required for successful amplification. DNA profiles were successfully generated from most of the samples of various available brands of lipsticks and blush sticks. This is the first study proving that DNA profiles can be generated from various lip and face cosmetics.

Keywords: Forensic Science, Cosmetics, Lipstick, Blush stick, DNA analysis, STR profiling, Human Identification

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دور أصابع أحمر الشفاه وأحمر الخدود في تحديد السمات الوراثية والهوية البشرية

المستخلص

إن الأهداف الأساسية للدراسة الحالية هي تحديد السمات الوراثية للإنسان من خلال بعض أدوات مستحضرات التجميل المستخدمة مثل أصابع أحمر الشفاه، وأصابع أحمر الخدود، وقد أجريت هذه الدراسة على العلامات التجارية المتاحة في باكستان. أُختير ما مجموعه 12 ماركة عالمية ومحلية من أصابع أحمر الشفاه وأحمر الخدود. وقد تم تطبيق أحمر الشفاه وأحمر الخدود على عدد عشرين متطوعة كانت أعمارهن تتراوح ما بين 21-30 سنة،

ويتمتعن بصحة جيدة. جُمعت العينات من خلال مسح رؤوس أحمر الشفاه وأحمر الخدود المستخدمة بواسطة مسحة قطنية معقمة وجافة. ثم أجريت عملية التحليل النوعي والكمي من خلال تقنية Real Time Polymerase Chain Reaction (RT-PCR) بواسطة جهاز التقدير الكمي للحمض النووي Real Time PCR ABI™ 7500 وذلك باستخدام طقم التقدير الكمي Quantifiler® Duo DNA kit.

تم تكثير عدد 16 موقع وراثي للبصمة الوراثية (التتابعات القصيرة المتكررة Short Tandem Repeats (STRs)) للعينات بأطقم التكثير AmpFlSTR® Identifiler® PCR amplification kit، باستخدام جهاز التدوير الحراري Thermocycler ABI 9700.

أُجريت عملية الهجرة الكهربية للمقاطع المُكثرة من الحمض النووي باستخدام جهاز Genetic Analyzer Applied Biosystems 3130™، كما تم تحديد السمات الوراثية للعينات باستخدام البرنامج الحاسوبي GeneMapper®ID-X الاصدار رقم 1.0.

أظهرت نتائج التقدير الكمي أن كمية الحمض النووي المستخلصة من عينات أصابع أحمر الشفاه أكبر من تلك المستخلصة من عينات أصابع أحمر الخدود، كما أظهرت النتائج وجود تثبيط فقط في 16% من عينات التجميل المستخدمة في الدراسة.

وقد كانت السمات الوراثية التي تم الحصول عليها (ظُهِّرت) من جميع عينات أصابع أحمر الخدود أكثر وضوحاً مقارنة مع عينات أصابع أحمر الشفاه، حيث لم يتم الحصول على السمات الوراثية من عينة واحدة من عينات أصابع أحمر الخدود، حيث كانت كمية الحمض النووي المستخلصة منها تساوي (0.001 نانوجرام/ميكرولتر)، بينما لم تظهر السمات الوراثية لأربع عينات من أصابع أحمر الشفاه، حيث كانت كمية الحمض النووي المستخلصة منها تتراوح ما بين (0.001-0.003 نانوجرام / ميكرولتر) وذلك لأن كمية الحمض النووي المستخلصة من هذه العينات كانت أقل من الكمية المطلوبة لإجراء عملية تكثير ناجحة.

تم الحصول على السمات الوراثية لمعظم العينات من مختلف العلامات التجارية المتاحة من أحمر الشفاه وأحمر الخدود. هذه الدراسة هي أول دراسة تثبت أنه يمكن الحصول على السمات الوراثية للحمض النووي من مختلف عينات مستحضرات تجميل الشفاه والوجه المستخدمة في باكستان.

الكلمات المفتاحية: علم الأدلة الجنائية، مستحضرات التجميل، اصبع أحمر شفاه، اصبع أحمر خدود، تحليل الحمض النووي، تحديد السمات الوراثية، تحديد الهوية البشرية.

1. Introduction

In the modern era, cosmetics are widely used across the globe by various age groups of both genders. Cosmetics are used to alter the appearance of a person by hiding or highlighting certain features. Among many cosmetics, lipsticks and blush sticks are very common products in use. According to Harris Interactive (2014), the consumption of lipsticks and blush sticks represents 35% of the total consumption of cosmetics in the USA [1].

Blush sticks and lipsticks are applied directly onto the skin. Blush sticks are used to hide spots and to add colour. Lipsticks are used to colour and shine the lips. Blush sticks and lipsticks are generally available in the form of bars and are directly applied to the face. A single bar can be used several times by the same consumer and is a good potential source of DNA.

All body fluids like blood, semen, and saliva and body tissues like hair, nails, etc. are common sources of forensic evidence to perform DNA analysis [2]. However, in routine practice, blood or buccal swabs are used as standard reference samples for genetic profiling. Sometimes these standard samples are not available for comparison of DNA profiles. In such a scenario, nontraditional samples may be used as reference samples [4]. Ideally, any item which comes into contact with the human body can be used as a potential source of DNA, and that can then be used for genetic profiling. These items include clothing, shaving razors, and toothbrushes, etc. Highly sensitive and validated methods are developed to generate DNA profiles from such items [3]. These items can be used as the only available reference sample in cases of missing persons, mass disaster victim identification, and homicide.

Some studies have shown that approximately 0.4 million skin cells are shed by a person per day [5-6]. So when these cosmetics are applied onto the skin, these cells get attached onto the bars of lipstick and blush sticks. These cells can therefore be a good source of DNA to generate the profile of the user.

These cosmetics are composed of many components i.e. absorption base, castor oil, glyceryl monostearate, ceresin, beeswax yellow, carnauba, ozokerite, diethylene glycol monostearate, bromo acid, isopropyl palmitate, and color lakes. The exact formulation of these cosmetics is not known because it is patented by the manufacturer. Water soluble dyes are after used in these preparations to stain the skin. Water soluble colors are frequently used in these cosmetics along with the addition of small quantities of hygroscopic material in order to avoid evaporation. Sorbitol, glycol and glycerol are commonly used, which also contributes to darken the surface of creams [7]. These agents

along with certain dyes used in these cosmetics may cause inhibition in PCR and genotyping reactions [8]. These inhibitors may bind to polymerase enzyme and hinder its functionality. The dyes are also an important component which may cause problems during quantitation and genotyping [9].

The main objective of the current study is to generate human DNA profiles from used lipsticks and blush sticks of various brands available in Pakistan. The study also establishes the importance of these cosmetics as physical evidence and reference material for human identification purposes.

2. Materials and Methods

2.1 Sample Collection

In this study, 12 international and local brands of lipsticks and blush sticks are used to generate DNA profiles (Table-1). The cosmetics were used by 12 different healthy female volunteers (aged between 18-32) on their face and lips. The lipstick and blush stick bars were swabbed before being applied to the lips and faces of volunteers, and these swabs were considered as negative controls. The used lipstick and blush stick bars were swabbed and subjected to a DNA extraction process.

2.2 DNA Extraction and Quantification

DNA from the swabs was obtained by organic extraction using Phenol:chloroform:isoamyl alcohol with a ratio of 25:24:1 [10]. The quantification of DNA samples was performed on ABI Real Time PCR 7500 using the QuantifilerTM Human DNA Quantification kit (Applied Biosystems, Foster City, USA) following the manufacturer's recommendations [11]. The extracted DNA samples were stored at -20 °C. Dilution of extracted DNA samples was done by using sterilized deionized water.

According to the manufacturer's protocol, a high value of CT for internal positive control in a sample was considered as inhibition. The samples showing the inhibition were cleaned with affinity resin thiopropyl sepharose 6B and used for STR profiling [10].

Table 1- *Brands used in the current study*

International Brands of Cosmetics	
Jordana®	California, USA
NYX®	Michigan, United States
Bobbi Brown®	New York, United States
Rivaj®	London, UK
Karaja®	Mozzo, ITALY
Avon®	Northampton, UK
Diana of London®	London, UK
Pakistani Brands of Cosmetics	
Atiqa Odho™	Karachi, Pakistan
Genny™	Karachi, Pakistan
Sweet Touch®	Karachi, Pakistan
Christine™	Lahore, Pakistan
Medora®	Lahore, Pakistan

2.3 DNA Profiling

The amplification was done by using an AmpFlSTR® Identifiler™ PCR Amplification Kit (Applied Biosystems, Foster City, USA) according to the recommendations of the manufacturer, using 28 cycles [13]. This kit analyzes 16 loci i.e. fifteen autosomal short tandem repeat (STR) loci and an Amelogenin gene locus (gende-based marker). The DNA sample (9947A), given in the amplification kit, was used as positive control. GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) was used to amplify the STR loci. The amplified alleles were analyzed on an ABI 3130 Capillary Electrophoresis Genetic Analyzer [13].

For genotyping, 8.7 μL HiDi Formamide, 1 μL allelic ladder and 0.3 μL Liz-600 were added to 1 μL of isolated DNA. This mixture was run on ABI Genetic Analyzer 3130 for an injection time of 5 sec at 3kV. The results were analyzed using GeneMapper® ID-X Software version 1.0 (Applied Biosystems, Foster City, CA US). The minimum peak height threshold was 50 RFU (relative fluorescent units) while no maximum RFU value was applied to interpret results. DNA profiles were obtained from the reference buccal swabs taken from the female volunteers. The reference profiles were used to compare with the DNA profiles obtained from experimental samples.

3. Results

The reference buccal swabs from volunteers generated complete DNA profiles. A new lipstick and blush stick were purchased and swabbed before applying on lips or face. These swabs were considered as negative controls and profiled along with the whole batch of samples. No human profile was obtained from negative controls.

Nearly all samples taken from lipsticks and blush sticks of various brands yielded DNA in real-time PCR reaction. Table-2 shows the CT value of internal positive control

Table 2- *The CT values representing the presence of inhibitors in DNA samples extracted from lipsticks and blush sticks of various brands*

Sr. No	Cosmetics Brand	Threshold cycle for log phase (CT Value)**	
		Blush Stick	Lipstick
1	Jordana®	26.5	27.0
3	Bobbi Brown®	27.0	26.7
4	Rivaj®	30.5	33.2
5	Karaja®	28.3	28.0
6	Avon®	26.9	27.4
7	Diana of London®	26.5	27.0
8	Christine™	27.3	27.8
9	Medora®	28.7	29.5
10	Atiqa Odho™	30.7	33.7
11	Genny™	27.8	28.0
12	Sweet Touch®	27.5	30.1
2	NYX®	26.7	26.8

** *each quantification value is the average of duplicate runs.*

(IPC) in each samples which represents the inhibition during real-time quantification reaction. In positive control, internal positive control (IPC) requires 26 reactions to reach the early log phase of real time PCR reaction i.e. CT value is 26. So any increase in CT value represents inhibition in reaction. A total of four samples taken from lipsticks and blush sticks of these brands exhibited inhibition in real-time PCR. Samples from lipsticks and blush sticks of Rivaj® and Atiqa Odho™ showed inhibition during real time PCR quantification reaction. Samples taken from the blush sticks of Atiqa Odho™ and Rivaj® required more than 30 cycles in order to reach the log phase in real-time PCR reaction. Negative controls from lipsticks and blush sticks of Rivaj® and Atiqa Odho™ also demonstrated inhibition during real-time PCR reaction (Table-2).

DNA was obtained from all the samples taken from both kinds of cosmetics of various brands. The concentration of DNA obtained from blush sticks of different cosmetics varied greatly. High yields of DNA were obtained from samples from blush sticks of Chistine, NYX® and Genny™ , which were 0.042, 0.023 and 0.061 ng/μL, respectively. Low quantities of DNA were obtained from the blush sticks of Rivaj® and Diana of London®.

Good quality and quantity of DNA was obtained from all blush sticks and lipstick samples with the exception of one sample obtained from lipstick. Table-3 indicates that partial DNA profiles were obtained from blush stick samples of Bobbi Brown®, Diana of London®, Medora® and Sweet Touch®. Full DNA profiles were obtained from blush stick samples of Jordana®, NYX®, Karaja®, Avon®, Christine™ and Genny™ (Table-3). While from the lipstick samples, full DNA profiles were obtained from only four samples i.e. Jordana®, NYX®, Karaja® and Christine™ (Table-4).

These samples did not show any obvious fluorescent artifact during typing. Heterozygous allelic imbalance was observed in a few samples from both lipsticks and blush sticks. The presence of inhibitors and quality of isolated DNA can be analyzed by determining the heterozygous allelic imbalance. In order to determine the heterozygous allelic imbalance, sister alleles on a heterozygous locus were considered imbalanced if the height of peaks were -70% of each other [14]. The results demonstrated that Pakistani cosmetics brands that yielded profiles have shown heterozygous peak imbalance in at least one loci. Blush sticks of Bobby Brown, Karaja® and Diana of London® yielded

Table 3- *Concentration of DNA recovered and genotyping results from blush stick samples of tested cosmetic brands*

Sr. No	Cosmetics Brand	DNA Concentration (ng/μL)	DNA Profiling (No. of STR loci obtained)
1	Jordana®	0.009	Full Profile
2	NYX®	0.030	Full Profile
3	Bobbi Brown®	0.004	Partial Profile (10)
4	Rivaj®	0.001	No Profile
5	Karaja®	0.023	Full Profile
6	Avon®	0.027	Full Profile
7	Diana of London®	0.003	Partial Profile (14)
8	Christine™	0.042	Full Profile
9	Medora®	0.005	Partial Profile (15)
10	Atiqa Odho™	0.004	Partial Profile (3+X)
11	Genny™	0.061	Full Profile
12	Sweet Touch®	0.007	Partial Profile (11)

Table 4- *Concentration of DNA recovered and genotyping results from lipstick samples of various cosmetic brands*

Sr. No	Cosmetics Brand	DNA Concentration (ng/μL)	DNA Profiling (No. of STR loci obtained)
1	Jordana®	0.015	Full Profile
2	NYX®	0.007	Full Profile
3	Bobbi Brown®	0.002	Partial Profile (2+X)
4	Rivaj®	0.001	No Profile
5	Karaja®	0.065	Full Profile
6	Avon®	0.021	Partial Profile (12)
7	Diana of London®	0.009	Partial Profile (15)
8	Christine™	0.023	Full Profile
9	Medora®	0.002	Partial Profile (14)
10	Atiqa Odho™	0.001	No Profile
11	Genny™	0.002	No Profile
12	Sweet Touch®	0.003	No Profile

heterozygous imbalance on 6 loci in each case. Heterozygous peak imbalance was observed on four loci in lipsticks of Bobbi Brown®, Diana of London® and Avon®.

In lipstick samples, the average yield of DNA was less than the amount of DNA from blush stick samples. The amount of DNA extracted from blush sticks was variable among different brands. Table-4 shows that among the lipstick samples, the maximum quantity of DNA obtained was from a sample of Christine™ , which was 0.023 ng/μL, while the minimum quantity of DNA was extracted from a lipstick sample of Atiqa Odho™ .

No profile was obtained from lipstick samples of Rivaj®, Atiqa Odho™ , Genny™ and Sweet Touch®. These samples showed inhibition in RT-PCR (Table-1). The quantity of DNA in all the samples was very low, which resulted in a failure to generate complete profiles. In a Bobbi Brown® lipstick sample, a partial DNA profile was obtained containing 2 autosomal loci and one amelogenin locus (X). More than 10 loci were typed in samples taken from lipsticks of Karaja®, Avon® and Diana of London®.

4. Discussion

Cosmetics have gained acceptance as a source of DNA profiles for human identification [8-9]. The present study was aimed at the use of lipsticks and blush sticks for STR profiling. STR profiling has become the most popular and frequently used technique for human identification in forensic investigation and in the identification of missing persons and victims of mass destruction [15].

The presence of PCR inhibitors i.e., castor oil, glycerol, ceresin, beeswax, carnauba, ozokerite, glycol, bromo acid, color lakes etc. in the samples can be detected by a change in the cycle threshold (CT) values for the internal positive control monitored during real-time quantification. Few samples have shown inhibition during this reaction. The amount of samples used for quantification was not an obstacle in the process of DNA quantification. This problem has been encountered due to the presence of a few components of cosmetics which caused inhibition in DNA quantitation, even if a large quantity of DNA is present in the sample [9, 15]. The local brands (Pakistani brands) were not found to be good sources of DNA to generate STR profiles. Among these, no profile was generated in 60% of these local brands. Nearly 33% of these samples generated full profiles and 50% of samples could not be used for identification. Among multinational brands, 50%

of lipstick samples were successfully genotyped. Overall, the yield of these samples was good and profiles were generated from low quantities of DNA; 71% of profiles were effectively used for human identification [16-17]. These profiles may be obtained from the cells that adhere to the surface of a used lipstick or blush stick, which might be shifted from the user to the cosmetics [18]. These cosmetics are made up of different constituents that may affect any of the processes of DNA analysis. One obstacle that may occur during genotyping is heterozygous peak imbalance. Such heterologous peak imbalance was observed in 25% of lipstick and 16 % of blush stick samples, and these findings are consistent with early reports [8, 19]. However, the heterozygous peak imbalance was not intense enough to prevent the interpretation of profiles. These cosmetics contained a variety of dyes, waxes, and other constituent molecules which can influence the efficiency of the STR profiling. It is very difficult to attribute these impediments to any particular component of these cosmetics [19].

Unlike lipsticks, blush sticks are not commonly used for DNA profiling. This study showed that blush sticks are also a good source of DNA that can be used for human identification. Approximately 50% of blush stick samples generated full profiles [Table-3-4]. Among all samples, 91% samples could be successfully used for genetic identity with high probability when compared with the reference DNA profiles. Our results indicated that the blush sticks present at a crime scene or related to any missing person can be used for STR profiling which would help for identification purposes.

5. Conclusion

Any evidence for forensic casework is critical. Sometimes, a minor piece of evidence can play a key role in solving a crime. It is very important to establish techniques that could make use of such vital evidence and generate conclusive information from them. Cosmetics are personal items and can be used for DNA profiling of their users. There are certain challenges associated with the chemical composition of these items to generate STR profiles. They include inhibition in PCR reactions that eventually effects genotyping reaction. These issues needed to be investigated and resolved so that such items of evidence can be effectively used to generate DNA profiles using STR markers. The present study has found that used lipsticks and blush sticks of international and national (Pakistani) brands can be effectively used to generate autosomal DNA profiles. Complete STR profiles were generated from most of the samples. Partial DNA profiles were obtained from a few lipstick and blush stick samples. These partial profiles can be used for human identification with high probability values. Further studies are required in order to establish a protocol that can extract a good quality and quantity of DNA from cosmetics so that a conclusive result could be obtained from available evidence. It is also recommended to conduct more studies with other cosmetics so that these can be used successfully for forensic purposes.

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