

## Original Articles

# Usefulness of a buccal smear in the initial assessment of a baby with a disorder of sex development

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## Abstract

**Introduction:** A newborn with a disorder of sex development (DSD) has ambiguous genitalia and thus, it is difficult to identify the sex of such a baby at birth. Early identification is essential for psychological, social and medical reasons. Therefore a karyotype is mandatory but the result takes time whereas a buccal smear result for Barr bodies is available within a few hours.

**Objective:** Assess the usefulness of a buccal smear as an initial investigation for genetic assessment of a baby with a DSD.

**Method:** A buccal smear and karyotype were done on all babies with a DSD referred to a ward at the Lady Ridgeway Hospital, Sri Lanka over 3 years from November 2008. A smear was made from the buccal mucosa and 100 squamous cells were examined for the presence of Barr bodies. Control samples were taken from their mothers. Barr bodies will be present in  $\geq 25$  cells if the baby is 46, XX and there will be no Barr bodies in 46, XY.

**Results:** Buccal smear result corresponded to the karyotype in 23 of 33 (70%) patients. The smears were inconclusive in 5 and were negative in 4 with 45, X/46, XY. The strength of agreement between a buccal smear and karyotype in assessing sex was 'moderate' (kappa 0.571,  $p=0.001$ ). All buccal smear results were available in 24-48 hours and 73% of the karyotype results after 4 weeks.

**Conclusions:** This is a useful initial investigation for genetic assessment prior to a karyotype in a baby

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with a DSD. Sex chromosome mosaicism cannot be identified by this method.

(Key words: Disorders of sex development, karyotype, Barr bodies in a buccal smear for genetic assessment)

## Introduction

The very first question asked by parents and family members at the birth of a newborn is the sex of the baby. Thus, presence of sexual ambiguity in the newborn is extremely distressing to everyone concerned.

Identification of the sex of a newborn with a disorder of sex development (DSD) is difficult when the appearance of the external genitalia is ambiguous. There are several conditions resulting in these disorders and a disease such as the salt-wasting form of congenital adrenal hyperplasia is fatal unless treated promptly and adequately<sup>1</sup>. At times it is impossible to identify the sex clinically and it is not advisable to guess the sex of such a baby<sup>2,3</sup>. Hence, early identification of the sex is essential, irrespective of the cause of the DSD, to counsel the parents, register the birth and commence treatment and discuss the long-term implications of life-threatening disorders.

A karyotype is mandatory to confirm the sex of a baby. However, this investigation is not free to the parents and it takes time for the result to be available whereas a buccal smear examination for Barr bodies (sex chromatin) is free and the result is available in a few hours. Barr bodies are formed by the inactivated X chromosome and appear as a black area on the nuclear membrane<sup>4</sup>. For Barr bodies to be present there should be 2 X chromosomes in the genotype.

## Objective

To assess the usefulness of a buccal smear as an initial investigation for genetic assessment of a baby with a DSD

## Method

All children with ambiguous genitalia referred to the University Paediatric Unit at the Lady Ridgeway Hospital (LRH), Colombo, were included in the study which was done over 3 years from November 2008. Informed verbal consent was taken from the mothers and the study was approved by the Ethical Review Committee of LRH.

Apart from the biochemical and radiological investigations that are indicated in a baby with a DSD, a karyotype and buccal smear were performed on all the patients.

A smear was made from the buccal mucosa and after adding one drop of the reagent Orcein in Acetic Acid, 100 squamous cells were examined for the presence of Barr bodies by a Consultant Histopathologist. Control smears were taken from their mothers as the 'control' should be a person who has had a child. In a 46, XX child,  $\geq 25$  cells will be positive for Barr bodies (sex-chromatin positive smear) whereas there will be no Barr bodies in a 46, XY.

The comparison of these two investigations was assessed in terms of agreement between them, using the kappa coefficient<sup>5</sup>. The fact that karyotyping classifies the sample into distinctly different groups did not allow us to assess the usefulness of buccal smears compared to karyotyping in terms of sensitivity and specificity.

## Results

Samples from the 33 patients referred to the ward were analyzed. The buccal smear result corresponded to the karyotype in 23 (70%) patients. Smears were inconclusive in 5 (3 with 46, XX and 2 with 46, XY) and were negative in 4 with 45,X/46,XY (Table 1).

**Table 1: Strength of agreement of buccal smear result with karyotype**

Buccal smear	Karyotype			
	46,XX n=10	46,XY n=18	45,X/ 46,XY n=4	46,XX/ 47,XXY n=1
Smear positive	7	0	0	0
Smear negative	0	16	4	1
Inconclusive smear	3	2	0	0

*Kappa 0.571, p=0.001*

All buccal smear results were available within 24-48 hours while in the majority (24/33→73%) the karyotype results were available after 4 weeks whereas minimum time taken was 2 weeks (Table 2).

**Table 2: Time taken for karyotype result**

Time taken for report	Number of samples
2 weeks	06
2 – 4 weeks	03
4 – 6 weeks	19
6 – 8 weeks	01
> 2 months	04

The strength of agreement between the buccal smear and karyotype using the kappa coefficient was 'Moderate' – kappa 0.571, p=0.001 (kappa value ranges from <0.2 to 1.0: Poor to very good)<sup>5</sup>.

## Discussion

With the advancement in cytogenetic studies in the recent past, demonstrating sex-chromatin in a buccal smear is not a favoured method of identifying the sex of an individual. Karyotyping can be done in our country but it is not free to the parents and as demonstrated, the result takes a significantly long time to be available to the clinician. Identification of the SRY gene by the PCR method or FISH technique, the result of which takes time to be available, can also be done. But this has to be followed by a formal karyotype and the total cost of all the investigations is too much to bear for most parents of babies in state-sector hospitals.

Whatever the underlying condition is in a baby with a DSD, the parents need to be informed of the probable sex of their baby as soon as possible after birth for psychological, social and medical reasons. Therefore, although not the ideal, in a resource-limited country where parents are faced with financial constraints, a buccal smear examination is useful as an initial investigation to inform the parents the probable sex of the baby and commence the process of counseling regarding the implications. Although life saving treatment is started when salt-wasting CAH is suspected on clinical and biochemical grounds as the cause of the DSD, the parents have to be informed as early as possible whether their baby is a girl or a boy. This is essential to discuss the gender-dependent long-term implications of the condition.

When investigating reproductive function this method had not been useful as conditions such as Turner or Klinefelter syndrome which are important causes of sub-fertility, could not be identified by this

method as well as autosomal constitution, Y chromosome or structural abnormalities of the X chromosome<sup>6</sup>. Although this had been used in the past for gender verification in athletes, it is not advocated now<sup>7</sup>. Buccal smear examination had been reported as a useful, simple method complementing prenatal chromosomal diagnosis on amniotic fluid cells<sup>8</sup>.

## Conclusions

We found this method of sex determination a useful *initial* investigation in a baby with a DSD as the result is available within a few hours and the process of counseling could be started soon thereafter. Sex chromosome mosaicism cannot be identified by this method. Although useful, we wish to emphasize that this investigation *does not* replace a karyotype for definitive identification of the sex of a baby.

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