DOI: 10.14260/jemds/2015/2063

ORIGINAL ARTICLE

A STUDY OF SEX CHEROMATIN FORM BUCCAL SMEAR

R. Shankar[1], P. Brahmaji Master[2], L. C. Obulesu[3], Y. K. C. Rangiah[4]

HOW TO CITE THIS ARTICLE:
R. Shankar, P. Brahmaji Master, L. C. Obulesu, Y. K. C. Rangiah. "A Study of Sex Cheromatin Form Buccal Smear". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 83, October 15; Page: 14497-14503, DOI: 10.14260/jemds/2015/2063

ABSTRACT: The determination of sex of an individual is an important subject in Forensic Medicine and Criminology and in Civil Law. The determination of sex is necessary in both living and the dead bodies. The expert opinion of the medico legal specialist regarding positive identification of an individual will be required for the following reasons. For the identification of the sex of individual whether living or dead, For deciding cases relating to legitimacy, divorce, paternity, affiliation, marriage, education, impotence, right to disposal of property, and in intersex condition & in cases of concealed sex. Determination of sex will be done by presumptive, probable and certain signs of sex. Sex chromatin is a planoconvex mass of about 1 micron in diameter lying near nuclear membrane-Barr body. In the buccal smear the percentage of the nuclei containing chromatin body ranges from 0 to 4 in males & 20n to 80 in females. In females neutrophil leucocytes contain a small nuclear attachment of drum stick form -David son body in up to 6% of cell. This is absent in males. Exact sex determination can be made by using a single specimen of buccal smear, saliva or hair follicle, by the combined treatment of quinacrinedi hydrochloride staining for Y chromosome which is seen as bright florescent body in the nuclei of male cell & florescent feulgen reaction using acriflavin Schiff reagent for X chromosomes, which is seen as bright yellow spot in the nuclei. The percentage of quanacrine positive bodies ranges from 45 to 80% in males, and 0 to 4% in females. With feulgen reaction technique florescent bodies are found in 50 to 70 % of cell in females, and 0 to 2% in males. Determination of sex chromatin pattern was done by examination of oral smears technique on 50 males and 50 females who have attended the Casualty of Government General Hospital, Ananthapuramu., A. P., in the Dept. of forensic Medicine from 17-06-2012 to 30-12-2014. Slides were prepared and stained by Giemsastaining and studied under oil immersion microscope for the presence of Barr bodies. In all 50 female slides the sex chromatin was positive and it varied from 20 to 52% in the age group of 12 years to above 60 years. The mean frequency of sex chromatin positive cells was found 40.14% with the standard deviation of 6.596. In all male buccal smears the sex chromatin was negative. The frequency of incidence of sex chromatin also been studied in different age groups. The lowest count was scored in the 15 to 19 age group and the highest percent was scored in 10 to 14 age group. An attempt has also made to compare the present study findings with the available previous studies on Sex chromatin.

KEYWORDS: Determination of sex, medico legal importance of sex, Barr bodies & Davidson bodies, sex chromatin & inter-sex.

INTRODUCTION: In the living person sex will be determined by physical examination except in cases of inter-sex. In the dead when the body is fresh determination of sex will be done by the means of physical examination, but difficulty arises when body is highly decomposed, when the murderer mutilates the body& obscure its identity, when identification of the is required in mass disasters like plane crashes, railway accidents, and natural calamities like floods and earth quakes, when buried bodies were exhumed and subjected for autopsy examination for various purposes.

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In some criminal cases like murder infanticide, abortion, where pieces of tissues were found, blood found adherent to the weapon, or knives, used for the purpose may be available for the examination from which precise identification of sex may be possible. When the skeletal remains only are available to determine the sex. Presumptive signs of sex for the determination of the sex: This is based on the outward appearance of individual, features of the face and presence and distribution of the hair and clothing' sworn, manner of behavior, voice. The adoption of a female attire by the males and females as males will be determined by examination of sex chromatin. Probable evidence of sex: In Males – Absence of breast tissue and the presence of penis and testicles. In Female – Presence of developed breast and vagina and the uterus. Certain evidence of sex in male: Presence of testis and prostate and nuclear sex. In Female Presence of vagina, ovaries and uterus and positive nuclear sex. Determination of sex in the Inter- sex persons- sex determination in the living inter sex state can be done by sex chromatin examination.

Davidson (1960) divided the congenital intersex in four groups: 1. Gonadal agenesis: In this condition the lack of development of gonads & secondary sexual characters & testis or ovaries has never developed. The nuclear sexing is negative. In this condition gonads are absent as a consequence to genital ducts - do not developed. Genital ducts do not develop because of the absence of Gonadal precursors. 2. Gonadal digenesis: gonads or sex organs are present but fail to develop during puberty; these are 2 types of medico legal importance. A). Klinefelter's syndrome: In his condition the anatomic structure is male but the nuclear sexing is female. The sex chromosome pattern is XXY (47 chromosomes) It is diagnosed when there is delay in onset of puberty, Gynaecomastia, azoospermia, low levels of testosterone, testicular atrophy, hyper gonadotropic hypo gonadism-Defective development of testis or ovaries and excess pituitary gonadotropin secretion will be present. In this syndrome the anatomical structure is female but the nuclear sexing is male.

It is diagnosed by lack of development of primary and secondary sexual characteristics, Short stature, webbed neck, sealed chest, wide set –nipples, coarctation of aorta, renal defects. B) Turners Syndrome: in this condition the external sexual structures/morphology is that of female but primary &secondary sexual features will not develop. Amenorrhea, sterility, short stature, high urinary gonadotropins will be present. Nuclear sexing is XO & and the total number of chromosomes is 45 instead 46.cause will be defective polarization during cell division in the process of development of spermatozoa. At the puberty the testis or ovaries fails to develop. 3). True hermaphroditism or Bisexuality: in this condition ovary or testicles or two testis are present in the external genitalia of both sexes the somatic sex chromatin may be male or female. 4) Pseudoheramaphroditism: In this condition gonadal tissue of only one sex is seen internally but external appearance is of the opposite sex.[1]

a) Male pseudo hermaphroditism: Nuclear sex is XY but sex organs and sexual characteristics deviate to female form because of testicular feminization. It is characterized by female external genitalia, normal size breast. The testis is in the abdomen or the inguinal canal, 5a Reductase deficiency occurs.
b) Female pseudo hermaphroditism: Nuclear sex is XX, but the sexual characters of male are seen due to adrenal hyperplasia. 21 Hydroxylase deficiency will be present.

Barr body: in 1949 Barr and Bertram noticed a jextanuclear chromatin in the nuclei of all female cells. Subsequently it was named as sex chromatin .presence of sex chromatin in the cells is called chromatin positive and its absence called as chromatin negative.

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This chromatin body is absent male cells but may appear in certain types of inter-sex where the external sex appears at first to be the male.[2]

Morphology of sex chromatin: in most of the cells of the nuclei the form of triangle or it seems to be built up of two small rod lets (klingera spherical form is a drumstick in polymorph nuclear leucocytes (Mittwoch, 1967 and Tolks Dorf, 1974). The sex chromatin is usually located lying flattened against the inner surface of the nuclear membrane, measuring 0.8 X 1.1 u in size with a range of 0.7 X 1.0 to 1.0 X 1.1 units. The position of the sex chromatin is within the nucleus periphery often attached to the inner wall of the nuclear membrane. In nerve cells it is most commonly situated in the center of the nucleus in relation to the nucleolus, [3]

Fluorescent Y body: The visualization of the Y chromosome by fluorescent staining techniques developed by Casperson and collaborators, Pearson et al (1970) demonstrated a small fluorescent body in the interphase cell from human males. Therefore from the tissue cells it is possible to determine the sex in doubtful cases and also from the tissue fragments, non-pregnant uterus, uterus containing fibroid tumors.it is proved that the sex chromatin can be determined in unpreserved, un fixed human tissues. Fluorescent Y bodies can be demonstrated in variety of cells including buccal mucosa, fibroblast, and lymphocytes (Pearson Et al 1970, Polani and Mutton1971), hair root (François et al 1971, spermatozoa (Summer et al 1971) and amniotic cells Rook et al 1971 Nelson and emery and papa et al 1970).[4]

Dixon and Torr (1956) experimented in foetal tissues depending on the nature of the environment in which the tissue has been placed and identified the sex chromatin from to 2 to 3 weeks after the death of the foetus.[5]

Skeletal Remains: Krogman (1962 studied the bones which possess secondary characteristics of pelvis, skull mandible, femur, sternum, scapula, and long bones of the hands. From the entire 100%, skull alone 90%, pelvis alone 95%, long bones alone 80%, skull and pelvis 98% sex will be determined.

Sex chromatin and nuclear sexing is one of the important methods used in the determination of the sex. Barr (1954), Moore and Barr's (1995) they have taken smears from the oral mucosa of the cheek. Out of 140 subjects 81 were male and 59 were female. The age of the subjects ranging to 2 days to 62 years. After smearing the slides, were fixed immediately in papaniculous fixative (Equal part of 95% alcohol and ether and stained with cresyl-echt-violet. They examined the slides under oil immersion. The sex chromatin was located in various position in relation to the periphery of the nucleus. It was the most definite and easier to recognize when at the periphery of the nucleus. Measurement of sex chromatin with the filer micrometer eyepiece gave a mean value of 0.7 X 1.2U. In the cases of male smears similar chromatin was not seen.

They concluded that with reasonable care that chromosomal sex can be diagnosed from smear from oral mucosa with little chance of error. Davidson in 1960 used to recover leucocytes from the dried blood; smears were prepared from the lysed blood on the slides, and fixed with 10% formal water and stained with lieshman or Giemsa stain. He observed that the neutrophil leucocytes were well preserved. Drumsticks (Davidson's bodies) were recognized in most of the blood smears.[6] He mentioned that through this method is very tedious and very useful method in determining sex in Forensic medicine.

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Trichology: The cells of the hair – root sheath also exhibit sex differences. Schmid introduced one technique to study sex chromatin in the root sheath. In this method the hair is pulled off & treated with aceto-orcain for approximately 30 seconds at 65degree centigrade. Then under magnifying glass the hair bulb is cue and cylinder shaped root sheath is separated from the shaft.

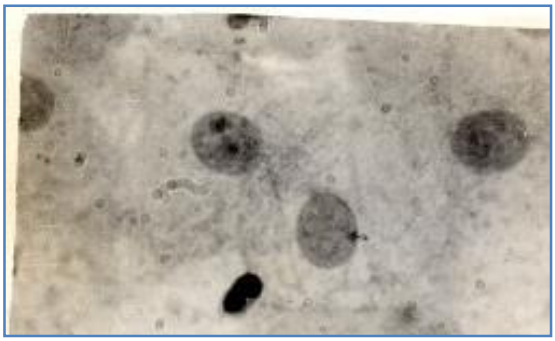
Then the material is kept under a cover sheath to obtain a single layer of cells. He found that sex chromatin can be studied by this method. Discovery of Barr bodies, Drumstick, Y bodies has made it possible to identify sex in some complicated forensic as well as Clinical cases.[4]

Experimental: Preparation of buccal smears: In this study epithelial cells are obtained by scraping the buccal mucosa with the small edge of the glass slide and round edged plastic spatula was used to avoid accidental injury to cheek. The material obtained by the first scraping is discarded. The material obtained by the first scraping is discarded. Second time healthier cells were obtained by scraping deeper layers of the epithelia. The material was spread over a small area of clean slide and allowed to dry for few minutes. After this cells were fixed in (Ether alcohol. One part ethyl ether, 1 part ethanol) for 20 minutes.

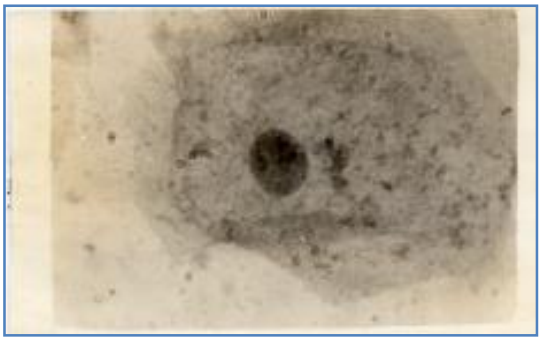
Staining: the slides were then passed through 7) % alcohol to water and rinsed in current water for five minutes and then the smears were hydrolyzed for 2 minutes at room temperature. Finally these smears were rinsed in distilled water and stained in Giemsa stain for 5 to 10 minutes.

Preparation of buffered Giemsa solution: Distilled water 100 ml, 0.1 M citric acid: 3ml, 0.2 M Na2 Hbo4: 3 ml, Methyl alcohol: 3ml, Giemsa blood stain: 5 ml (stock solution).

Picture.1: Showing Barr body (shown by arrow) in the buccal mucosal epithelial cell belongs to female individual.
Picture.2: Showing no sex chromatin in the nucleus of buccal mucosal epithelial cell belongs to male individual.



Picture: 1



Picture: 2

OBSERVATION AND RESULTS: In this present study we took 100 buccal smears, 50 male and 50 female from the patients who have attended the casualty of Government General hospital, Ananthapuramu from 17-06-2012 to 22-12-2014. Slides were prepared and stained with Giemsa staining and studied oil immersion micro scope for the presence of Barr bodies. When scoring Barr

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bodies in cells of the buccal mucosa, only peripherally located Barr bodies should be considered. Barr bodies cannot be confused with chromo centers as they are present at the periphery of the nuclear membrane. In all 50 females slides the sex chromatin was positive and it varied from 20 -50% in the age group of 12 years to above 60 years. The mean frequency of sex chromatin positive cells was found 40.14 % within the standard deviation of 6.59. In all male buccal smears sex chromatin was negative.

Evaluation of Barr bodies or Screening of sex chromatin: In this present study the criteria of the Hse et al 1967, has been employed for the nuclei examined for sex chromatin choosing the first. 100 encountered which "were flat, oval, un obscured and well stained with smooth, clearly defined unbroken nuclear membrane and finally dispersed nucleus chromatin. To consider sex chromatin positive nuclei meeting (these) criteria and to contain a deeply stained, clearly defined chromatin mass at least 1 u in it's in longest diameter and situated against the inner surface of the nuclear membrane.[7]

DISCUSSION: Sex can determine by studying the presence of sex chromatin in various tissue of the body, i.e. blood, skin, amniotic fluid, buccal mucosa. Among all the tissue cells, buccal mucosal scrapings for study of sex chromatin in the petechial cells are the best and simple technique. In this study the sex chromatin can be determined with ease, speed and accuracy. Sex determination from buccal smear was first demonstrated by Moore and Barr (1955) in Canada. Later Marbarzar et all in 1955, United States, and Dixon and Torr in 1956 made a study on it. Originally this method of sexing was used for diagnosis of chromosome anomalies during development and was limited clinical practice. Forensic interest in sex chromatin started largely with the work of Dixon and Torr in 1956-57.[5]

In the smear preparation of oral mucosa cells from female persons, only 25-50% the nuclear x- chromatin positive. This low % is certainly partly due to technical difficulties in producing smear preparations. Unfortunately tissues which are suitable for clinical studies from the oral mucosa have much lower frequencies(36-76%) when compared with the sex chromatin frequencies obtained from female foetal membrane was 96% and 85-95% from the neurons. Moore and Barr used cresol – echt-violet for staining the slides. Dixon and Torr used cresol violet. David son in 1960 introduced Giemsa staining for buccal smear study. The advantage of using this Giemsa stain was the stained cells were well preserved and Barr bodies can be easily and clearly recognized. For this advantage we have used Giemsa stain in this study. Presence of sex chromatin is thus a reliable and valuable index for sex determination.

In the present study it was observed that the chromatin in the frequency of 40.14% in all female cells with average sex chromatin varied from 20-52%, while none of the male cases showed sex chromatin. It is thus felt the absence of sex chromatin is not medico legally helpful but its presence is helpful to presume the sex of the female. The frequency of incidence of sex chromatin also been studied in different age groups. It is observed that sex chromatin positive cells range 36.002 to 48.00 in different age groups. The lowest count was scored in the 15-19 age group and the highest % was scored in 10- 14 age group. The present work is conforms to the work of the other authors to the age incidents.

However daily buccal smear from 42 female babies showed at the 3rd day the sex chromatin frequency rises and was comparable to that found in older females. This may be due to altered

metabolic processes at this time. A similar reduction in frequency of sex chromatin was also noticed in mothers during the day prior to deliver and the first day or so after words, and S.W. Smith at all(1963) have suggested that there may be a common etiology for the reduction in sex chromatin in mothers at partirution and in new- born infants. [8] The Barr body examination helpful in determination of the sex not only of the adult but also of the foetus in the utero. The exfoliated cells from the skin floating in the amniotic fluid can tapped, centrifuged and examined for Barr bodies. The technique has also got a vast clinical application in determining genetic disorders. (Sex linked disorders). The sexing of the foetus by amniocenteses has however assumed a sinister forensic significance in view of its misuse for committing female feticides. In view of the simplicity of technique and collection of samples, it is felt that sex chromatin examination of buccal smear could be consider a valuable tool for the determination sex of an individual.

CONCLUSION: The study of sex chromatin was undertaken with the sole idea of doing some humble contribution in the field of forensic medicine, with particular reference to the determination of sex from the buccal smear. Buccal sex chromatin counts vary widely in individuals from day- to day hour to hour, and it is easy to collect the samples from the individuals. The determination of sex chromatin is easy, within minutes we can determine the sex of the person or individual while compare the sex determination from individual while compare the sex determination from other methods like, sex chromatin from polymorph leukocytes, Hair root, and skin, and amniotic fluid. A single buccal sex chromatin count is therefore not suitable as a diagnostic ambiguous sex and further evaluation like chromosomal study, karyotyping is essential. In the present study an attempt has been made to report the sex chromatin frequency in 50 female individuals.

The study is undertaken in the department of Forensic Medicine, Government Govt Medical college, Anantapuramu from 17-06- 2012 to 22-12-2014. We have collected buccal smear samples in casualty, government general hospital, Anantapuramu, from the patient attended the casualty. Buccal smears were collected from 50 male and 50 female individuals. Smears were stained with Giemsa staining and studied for the presence of sex chromatin under oil immersion microscope. In 50 female individuals the sex chromatin was positive with an average of 40.14% of the cells showed sex chromatin mass. The sex chromatin varied from 20- 52% in the age group of 12 years to above 60 years. An attempt has also been made to compare the present findings available literature. In 50 male individuals no sex chromatin was present against the normal range of 4-7 % as shown by Guard.

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AUTHORS:

1. R. Shankar
2. P. Brahmaji Master
3. L. C. Obulesu
4. Y. K. C. Rangaiah

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Forensic Medicine, Kurnool Medical College, Kurnool, Ananthapuramu, Andhra Pradesh, India.
2. Associate Professor, Department of Forensic Medicine, Govt Medical College, Ananthapuramu, Andhra Pradesh State, India.
3. Professor, Department of Forensic Medicine, Govt Medical College, Ananthapuramu, Andhra Pradesh State, India.
4. Assistant Professor, Department of Forensic Medicine, Kurnool Medical College, Kurnool, Andhra Pradesh State, India.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. R. Shankar,
Department of Forensic Medicine,
Kurnool Medical College,
Kurnool, Andhra Pradesh State,
India-518001.
E-mail: drrshankarramavath2@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None

Date of Submission: 09/10/2015.
Date of Peer Review: 10/10/2015.
Date of Acceptance: 12/10/2015.
Date of Publishing: 14/10/2015.