Barr bodies in sex determination

Sex determination from pulpal tissue

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Forensic odontology is useful in identification of age and sex of patients. Sex of the individual can be determined based on the morphology of canines. Apart from this method, it can also be determined by using X and Y chromosomes in the cells which are inactive. X chromatin in its inactivated form is present as a mass against the nuclear membrane in females is known as Barr body as it was first named by Barr and Bertem (1949). These Barr bodies are present in 40% of females who are considered as chromatin positive and absent in males who are considered as chromatin negative. Similar to X chromosome, Y chromosome (F bodies) can also be studied for sex determination of males. Both X and Y chromosomes are found to be present during interphase of the cell cycle.

The authors have carried out this study was to determine the period of time after death upto which sex of the individual can be determined, depending on variation of temperature and humidity. In this study, a total of 100 cases were analyzed out of which 90 cases were selected from cadavers and 10 cases from patients undergoing treatment. Teeth involved in this study were canine and incisors. A group of 10 (fiveeach of male and female) was examined at various time intervals. Pulp tissue obtained from these teeth were smeared in two slides each of which is stained by H and E and quinacrine dihydrochloride for the study of X chromosome and Y chromosome, respectively.

It was concluded that sex determination using human pulp in cadavers is possible only up to a period of 4 weeks. For accurate diagnosis, Barr bodies should be more than 6% and F-bodies should be up to 4% until a period of 4 weeks since death. It is important to determine both the presence or absence of X and Y chromosomes in human pulp tissue because due to variations in temperature and humidity, pulp tissue undergoes putrefaction quickly which in turn gives negative results of either of sex chromatin.

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Role of Barr bodies obtained from oral smears in the determination of sex

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Various cytologic studies prove the presence of condensed deeply stained chromatin material in nuclei of female cats in 1940's which was later termed as Barr-body by Murray Barr. These cells found to be present only in females which can be used as a vital tool for determination of sex of the individual. Significance of presence of such densely stained material in nuclear material was given by Lyon, who suggested that inactivation of one of the X chromosome in each somatic cell occurs during the early embryonic development and named such process as lyonization. Simple techniques such as determination of sex from buccal smear weregiven by Moore and Barr.

Inactivated X chromosomes seen in female somatic cells are called Barr-bodies which are present adjacent to the nuclear membrane. Condensed state of densely stained chromatin signifies that in such cases DNA replication occurred at the later stage of S phase of cell cycle. They generally appear as basophilic structures with varying morphology which can be either spherical, rectangular, planoconvex, biconvex, or triangular measuring 0.8×1.1 microns. It also resembles various alphabetical letters in electron microscope as V, W, S, or X. Barr body can also be obtained from buccal smear, pulp tissue, vaginal smears, and hair follicle.

Since Barr bodies are present within nuclear material, special stains for nucleus such as papanicolaou stain, feulgen and guard stains, orcein, hematoxylin and eosin, cresyl violet, carbol fuschin, and fluorescent staining are used to visualize them. Under various pathological conditions, there are alternation in number and size of Barr-body, which produces negative results and produces difficulty in sex determination. Advanced techniques such as *in situ* hybridization, immunofluorescence method can be determination of the sex of the individual.

Sex determination from buccal mucosa scrapes

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Forensic dentistry prime concern of importance is to identify the sex of the individual. There are various methods dealt such as tooth, blood, hair, semen, and saliva containing cells from buccal mucosa. Although various methods are available one of the simplest and easiest method is to identify sex of the individual is from buccal mucosal scrapes. In this method, sex of the individual is identified using nuclear sex chromatin method.

According to this study, 200 samples were included out of which 100 each of males and females. Patients advised to rinse their mouth with mouthwashes before obtaining the samples. Samples were obtained using metal spatula from buccal mucosa where smeared in the frosted slides and then fixed in ethyl alcohol 95% for 10-15 min. Slides were then rinsed in distilled water and then stained with papanicolaou stain and mounted. Compound microscope under oil immersion was used to visualize the slides.

Results found to have 1.14% positivity for males and 39.29% positivity for females. Positivity for Barr-body in males is due to the inheritance of males to carry primary sex organs of both the sexes. But the range of positivity differed when compared with males, which is more significant in females. Papanicolaou staining is the gold standard staining method for cervical cancers smears because of its excellent staining capacity of nuclear substances. Thus, in a nutshell determination of sex of individual by Barr-body plays a vital role in identification; author concluded that it can be done by using papanicolaou staining due to its perfect nuclear staining, cost-effective, and time-consuming and also by viewing under compound microscope under oil immersion technique gives excellent and clear details for identification of Barr-body.

Sex chromatin in dental pulp. Performance of diagnosis test and gold standard generation

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Determination of sex is very important in forensic medicine. But very often, the available skeleton resource in forensic identification of sex of the individual becomes a challenging task owing to the deficiency of the substance for specimen analysis. In such cases, the tooth which remains intact can be of significance in forensic identification of sex of the individual. Barr and Bertram determined that there exists difference between male and female cells by presence of Barr body in females. Depending on these feature sex determination was done in this study.

This study included sample size of 40 individuals which was divided into two groups consisting of 20 men and 20 women between the ages of 24 and45 years. Tooth included in this study was healthy without any pathology. Extracted tooth was preserved in 5% formalin for 7 days and pulp tissue was obtained and paraffin blocks were made. Each of the section was cut of 5 μm thickness was cut and processed stained with hematoxylin and eosin and mounted. Slides were viewed using trinocular microscope C×21 under ×100 magnification. Around 50 cells were viewed in each plate and the presence of visible Barr body was considered positive for females.

The results obtained were 100% positive for females and negative for males which correlated with the literature that sex determination in dental pulp tissue can be done. Although difference of opinions from various authors regarding the time period up to which pulp can be used to determine sex of the individual, its finally concluded that depending on different geographic location and its influence on obtained specimens its varies. Only limitation for the use of this method was that if the alternations at the chromosome level occur in men and women with aneuploid, in such cases negative or false positive results are obtained.

Sex determination by observation of Barr body in teeth subjected to high temperatures

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Determination of sex in natural disasters is challenging task because obtained victims body are subjected to severe damage due to natural disasters. In such situation, identification of sex becomes difficult but can be overcome by using teeth as a vital tool for sex determination through the presence of Barr body in females. Therefore, this study was done to evaluate effect of influence of high temperature on pulp tissue and its ability after subjected to high temperature in determination of sex.

In vitro study of 50 teeth, 25 each of males and females were taken. Inclusion criteria for the selection of teeth in this study werehealthy teeth, extracted for the purpose of orthodontic treatment, prosthodontic treatment, and third molars for prophylactic purposes. Obtained teeth was placed in 10% formalin at a temperature of 34°C and relative humidity of 100%. Then teeth were subjected to varying degree of heat treatment from 200,400,600,800, and 1000°C in Wenzhou Shangtong instruments Co., Ltd (STYB) dewaxing oven model K955 with 10 teeth at each temperature. After this coronal pulp tissue was extracted and processed. Each tissue sections are cut at 7μm thickness and analyzed under a trinocular microscope olympus.

Results were positive for females in the tooth subjected to temperature until 400°C, whereas tooth subjected to further

high temperature it was not possible to find any viable tissue for analysis. This correlates to the fact that tooth is well-preserved within oral cavity so that only minimum heat dissipates to the tooth which makes dental pulp tissue to withstand and helps for the diagnosis of sex in forensic medicine.

Reliability of Davidson body to determine the nuclear sex of the individual: Interobserver variability between pathologists

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Individual identity in forensic odontology is important to clear out various medicolegal problems. Certain difficult situations when more degraded and decomposed samples are obtained only way for identifying the individual is by sex determination. Sex determination can be done by identifying sex chromosome which is unique. In such conditions, blood smear can be used to identify the sex. Barr-body in neutrophils of blood smear has specific appearance of chromatin appendage resembling drum-stick called Davidson body.

Sample sizes of 200 cases were included in the study 103 males and 97 females with inclusion criteria of nonsyndromic individuals. Peripheral blood smear were made and stained with Leishman stain. Two pathologists examined each slide individually and finally the obtained results were correlated with the phenotypic sex of the individual. Results were negative for Davidson body in males and positive for females.

Authors conclude from this study is that when samples obtained are very minimal or damaged lot, in such circumstances peripheral blood smear is the easy method to identify the sex of the individual. This method also is time-consuming, easy, and quick when blood sample is only way for identification.

Determination of sex by exfoliative cytology using acridine orange confocal microscopy: A short study

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Identity of an individual is one of the important perceptive in forensic dentistry. It becomes a challenging task when insufficient samples are obtained. In such conditions, nuclear sex determination of an individual paves way for identification of the individual. Nuclear sex chromatin can be demonstrated by various methods one such method is buccal smear method.

Individuals have the possibility of carrying the primary sex chromosomes of both the sexes, so this study was mainly done how to differentiate the expression of Barr-body in females and males in order to set criteria for identification. In this study, authors have considered sample size of 20 each of male and female. From each individual buccal smears were obtained by exfoliative cytology using flat wooden sticks. Then, the samples were fixed in 100% alcohol for 15 min and stained with acridine orange staining. Later, the slides were mounted and viewed under confocal microscope.

Results obtained found to have in males 0%-3% were Barr-body positive and in females 18%-72%. Acridine orange found to have proper staining property and when viewed with confocal microscope produces classical contrast needed for observation of cells without light dispersion. Thus, concluding from this study is that Barr-body found to be positive in both sexes with or without syndromes; it is needed to stain it appropriately and to visualize using proper microscope because Barr-body has the capacity to polarize.

Role of forensic odontologist in postmortem person identification

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Forensic odontology was first described by Keiser-Neilson in 1970, which plays important aid in identification of the individual. Natural teeth which resist various natural disasters can be used as reliable one for the forensic investigations. Previous historical review states that right from 66 A.D. to recent literature tooth has been used as key substances for identification of the individual because of its hard enamel core which resist various insult to it and also dental identification is simpler, easier, and quickest method of identification.

Patient who has both antemortum and postmortem data in detail identification of the individual becomes easier task by comparing each of the data obtained. In cases, were antemortum data aremissing or insufficient forensic investigations are needed. Various investigatory methods are available for identification such as anthropology, cheiloscopy, bite-mark analysis, rugoscopy, tooth prints, radiographs, dental DNA analysis, and so on. Each of the above technique has its own limitations such as difficult to obtain lip prints in carbonized bodies, bite marks shrink in few minutes after the incident has occurred, and so on. Tooth is the only substance able to withstand all the insults occurring to it and can be used for identification of individual under all circumstances.

Along with the forensic investigations, there are various factors such as identification of work place of the

individual, location of residence, and any genetic basics are considered because these factors has influence on the dental structures. Sex determination of an individual is equally important for identification of individual which is done by using pulp tissue. One method is by identifying Barr-body in females and other method is by identifying amelogenin gene by DNA polymerase chain reactionanalysis. Barr-body identification in pulp tissue is considered gold started for identification because its ability to resist the various disasters with its protective hard enamel core.

Thus concluding, along with forensic investigations, antemortum records also have equal importance for identification of the individual. Forensic odontology has a prime role in identification of the individual even in a critical situation where the obtained sample is severely damaged and decomposed.

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