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SEX DETERMINATION FROM PULPAL TISSUE

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ABSTRACT

It is a well known fact that the sex can be determined from the pulp tissue in living as well as dead, but up to what postmortem interval it can be determined accurately is still a matter of controversy amongst different authors. An attempt has been made in this regard, keeping in view the effect of environment i.e. Role of temperature and humidity on pulp tissue after the extraction of teeth.

KEY WORDS: Pulpal tissue, Sex Determination, Teeth, Odontology, Barr Bodies, F-bodies.

INTRODUCTION

The study of teeth reveals a lot concerning forensic medicine. Particularly it is useful in identification. Forensic Odontology tells us a lot about determination of age from eruption of teeth [1,2,3], and calcification of their roots below the age of 25 years. Boyd's incremental lines for a few months after birth [4] and Gustafson's method for age determination above 25 years [5]

In addition to determination of age sex can also be determined from the teeth. From the morphology of canines sex can be differentiated by studying the mesiodistal crown width, greatest crown length and canine separation width [6,7]

Sex can also be determined by the study of X & Y chromosomes in the cells which are not undergoing active division. Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp. After death it persists for variable periods depending upon the humidity and temperature in which tissue has remained. X chromatin and intra-nuclear structure is also known as Barr body as it was first discovered by Barr & Bertam (1949). It is present as a mass usually lying against the nuclear membrane in the females [8]

These Barr bodies are found in about 40% of female cells which are known as chromatin positive, and male cells are chromatin negative. It is found only in those cases in which more than one X chromosome is present [4]. Y chromosome can be studied in the cells during interphase by staining with Quinacrine mustard when Y chromosome will fluoresce more brightly and its presence conclusively indicates the Y chromosome and sex in positive cases is invariably male [9].

As temperature and humidity effect the putrefaction so must be the effect of temperature and humidity on the pulpal tissue. In the present work it has been tried to know that up to what time after death we can determine the sex accurately from the study of X & Y chromosomes keeping in view the variation of temperature and humidity.

MATERIAL & METHODS

A total of 100 cases comprising of 50 males and 50 females were studied. Ten cases (5 males and 5 females) were selected out of patients who came for treatment at Government Dental College, Amritsar. The remaining 90 cases (45 males and 45 females) were selected from numerous dead bodies which were brought for medicolegal autopsies to the Department of Forensic Medicine & Toxicology, Government Medical College, Amritsar, at random. Only those cases were studied in which the time since death was exactly known. The dead bodies in which preservation had been done were not included in the present study. In living persons the time of extraction of teeth was noted in the Oral Surgery Department at Government Dental College, Amritsar.

The temperature and humidity of the mortuary, where the dead bodies were received or the extracted teeth were kept were noted by standard Hygrometer consisting of dry and wet bulb thermometer.

The teeth were extracted from the jaw with the help of tooth extractor. The canines or incisors were examined without any distinction. The canines

or incisors were selected preferably as they were easy to break and contained a good amount of pulpal tissue. A group of ten teeth (one each from 5 male and 5 female cases) were examined at intervals as shown in the following schedule.

S. No.	Duration
1	1 – 6 hours
2	6 – 24 hours
3	24 – 48 hours
4	2 – 4 days
5	4 – 7 days
6	7 – 10 days
7	10 – 15 days
8	15 – 21 days
9	21 – 28 days
10	28 – 35 days

Teeth were extracted and allowed to undergo changes in the prevalent atmosphere of the mortuary and examined at intervals as per schedule of the study. Temperature and humidity were noted at the time of study.

Teeth were broken lengthwise by striking with a hammer on the lingual surface at the junction of the crown and the root. The whole of the pulp tissue was separated out of the pulp cavity with the help of needle and forceps and transferred to a conical tube containing normal saline. It was adequately washed in normal saline to remove any calcified bone or dentine particles. The tubes were covered with aluminum foil and transported to the Center for Genetic Disorders, Department of Human Genetics, Guru Nanak Dev University, Amritsar, for further processing.

The pulp tissue was then transferred to the dry and clean conical centrifuge tubes containing 5 ml. of fixative (3 Methanol: 1 Glacial acetic acid) and left as such for about half an hour to 24 hours for the fixation of the pulp cells. It was then crushed / teased with the glass rod sufficiently to isolate the pulp cells. A suspension thus obtained was centrifuged for 10 minutes at 1000 rpm. The supernatant was discarded, leaving behind the pellet in the centrifuge tube. 5ml of fresh fixative was then added to re-suspend the pellet and the process was repeated thrice till a clear suspension of the pulp cells was obtained.

Thin smears were prepared on chilled microscope slides of 1 mm thickness by the air drying method i.e. by dropping 2 –3 drops of the above suspension on the slide from a distance of inches to get a homogenous population of cells. Two smears were made from each suspension of the specimen; one slide was stained with Harris's Hemotoxylin and Eosin stain to study the Barr bodies. The second slide was stained with 5% Quinacrine dihydrochloride for the study of Y chromosome [10].

The first slide was examined under oil immersion lens of light microscope for the Barr bodies. One hundred cells were scanned. The data was collected, compiled, studied, and then analyzed. In the second slide, mounting was done with a few drops of buffer of Ph 5.6 and the slide covered with cover slips of 22 x 40 mm in size & No. 0 in thickness avoiding trapping of any air bubbles. The cover slip was then sealed with nail varnish and the slides were scanned under oil immersion lens (100X), using Carl Zeiss fluorescent microscope having HBO 200 as light source and BG-12 as the excitation filter and OG as barrier filter. Only those cells which contained the characteristic Y chromatin i.e. a brightly fluorescent spot attached to the nuclear membrane were counted as positive cells while those which did not show any such fluorescent spot were labeled as negative.

OBSERVATIONS

Table 1 - In females with the increase in the postmortem number of Barr bodies decreased and we can determine the sex up to about four weeks with certainty when we take in to consideration both Barr bodies and F-bodies. If we don't take into consideration F-bodies, this interval is reduced to 3 weeks. In females maximum number of F-bodies found was eight.

Table 2 - In males with certainty we can determine sex up to four weeks and study of Barr bodies did not increase this interval. Maximum number of Barr bodies in any male was found to be six.

Table 3 - For Barr bodies study temperature up to 35.6 °C is more suitable and if temperature will increase from this, the number of Barr bodies decrease in 15 – 21 days study period and optimum temperature was 28.6 °C to 35.6 °C. For F-bodies low temperature was more suitable but up to 35.6 °C. Temperature did not have much effect.

Table 4 - For Barr bodies –for humidity not

Table 1 – Incidence of sex chromatin in the female cases from the period 1-6 hours to 28-35 days

S. No.	Time Interval	Avg. Temp °C	Avg. RH %	No. of Barr-bodies in females		No. of F-bodies in females	
				Mean ± SD	Range	Mean ± SD	Range
1.	1-6 hrs	30.4	79.6	47.50 ± 4.43	42-52	0.75 ± 0.95	0-2
2.	6-24 hrs	26.6	85.7	34.75 ± 7.08	26-41	5.00 ± 2.16	3-8
3.	1-2 days	19.1	86.2	36.75 ± 2.75	35-42	2.25 ± 2.21	0-5
4.	2-4 days	21.4	82.7	31.50 ± 3.87	24-37	3.00 ± 0.81	2-4
5.	4-7 days	26.8	77.3	26.25 ± 4.03	22-31	3.00 ± 2.52	0-7
6.	7-10 days	24.0	85.5	25.00 ± 4.32	20-31	3.75 ± 1.70	2-7
7.	10-15 days	27.5	80.9	20.50 ± 2.64	18-24	3.50 ± 1.29	2-5
8.	15-21 days	34.5	63.3	15.00 ± 3.74	11-20	1.50 ± 0.57	1-3
9.	21-28 days	39.9	53.2	9.25 ± 2.62	8-13	0.50 ± 0.57	0-1
10.	28-35 days	41.4	51.4	3.00 ± 2.00	0-4	0.00 ± 0	0-0
Mean ± SD		27.6 ± 6.06	71.1 ± 10.75	24.92 ± 3.74	0-52	2.27 ± 1.30	0-8

Table 2 – Incidence of sex chromatin in the male cases from the period 1-6 hours to 28-35 days

S. No.	Time Interval	Avg. Temp °C	Avg. RH %	No. of F-bodies in males		No. of Barr-bodies in males	
				Mean ± SD	Range	Mean ± SD	Range
1.	1-6 hrs	32.2	56.0	76.25 ± 5.90	69-82	1.0 ± 0.81	0-2
2.	6-24 hrs	30.1	76.2	48.25 ± 9.58	33-61	3.5 ± 0.57	3-5
3.	1-2 days	28.8	86.7	44.00 ± 4.96	35-50	2.5 ± 2.38	0-5
4.	2-4 days	21.9	86.5	35.25 ± 3.94	31-40	3.25 ± 1.89	2-6
5.	4-7 days	18.0	81.8	42.20 ± 8.22	30-51	2.0 ± 2.16	0-5
6.	7-10 days	17.8	82.9	38.50 ± 9.39	27-42	0.75 ± 0.95	0-5
7.	10-15 days	19.7	81.1	34.00 ± 11.9	20-47	4.0 ± 1.82	3-6
8.	15-21 days	26.7	81.7	22.75 ± 3.86	15-28	2.5 ± 0.57	1-3
9.	21-28 days	27.6	75.1	13.25 ± 4.99	9-20	0.75 ± 0.95	0-2
10.	28-35 days	26.7	70.1	02.00 ± 2.30	0-4	1.00 ± 2.0	0-4
Mean ± SD		24.0 ± 5.58	78.8 ± 8.58	35.64 ± 6.59	0-82	2.12 ± 1.41	0-6

Table 3 – Effect of temperature on sex chromatin [Barr bodies and F-bodies] from period 1-6 hours to 28-35 days

Temp °C	1-6 hrs		6-24 hrs		1-2 days		2-4 days		4-7 days		7-10 days		10-15 days		15-21 days		21-28 days		28-35 days	
Range	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB
13.4–21.4	-	-	26	-	34.3	-	30.5	41.6	-	38.0	25	36.2	-	33.5	-	-	-	-	-	-
21.5–28.5	-	-	31	50	37.5	35	29.6	33.5	27	-	23.7	-	19.3	24.6	-	22.7	-	12.6	-	0
28.6–35.6	48	75	40.5	44	-	42.5	-	-	-	-	-	-	21.5	-	17	15	-	-	-	-
35.7–42.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.6	-	9	-	1.6	-

F=female; M=male; BB=Barr Body; FB=F-body

Table 4 – Effect of humidity on sex chromatin [Barr bodies and F-bodies] from period 1-6 hours to 28-35 days

Humidity %	1-6 hrs		6-24 hrs		1-2 days		2-4 days		4-7 days		7-10 days		10-15 days		15-21 days		21-28 days		28-35 days	
Range	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB	BB	FB
46.5–57.5	-	75	-	-	-	-	-	-	-	-	-	-	-	-	13	-	9.25	-	2.4	-
57.6–68.7	-	-	-	-	-	-	-	-	30	-	-	-	-	-	13	-	8.0	10	-	0
68.8–79.9	46	-	26	45.2	-	-	-	-	27.5	43.5	-	46	19	34.5	20	21.5	-	11.5	-	1.3
80.0–91.5	49.3	-	35	-	37.8	42.2	30	40.4	25	43	24	29.6	21	30.6	-	21.3	-	15	-	-

F=female; M=male; BB=Barr Body; FB=F-body

enough data was available to reach a conclusion, probably findings of Barr body is easier at humidity of 68.8 % – 79.9 %. For F-bodies – optimum humidity was 68.8 % - 79.9 % and with increase in humidity number of F-bodies decreased.

DISSCUSSION

Determination of X & Y chromatin

Mean percentage of human tooth pulp showing F-bodies in the males was found to be 35.64% and in females 2.27% which is almost similar to 30% and 4% respectively in the study by Seno and Ishizu [11].

Highest level of F-bodies 82% in this study is almost consistent with 84% of Whittaker et al [12]. Though their mean range of 17.4% - 51.2% in males, and 0 – 16% in females is different from 13.2% - 76.2% in males and 0% – 5% in females. Yet, these ranges are similar to those of Duffy et al [13] i.e. 37% - 75% in males and 0.9% - 4.6% in females. As far as Barr bodies are concerned according to Duffy at el [13] count of Barr bodies in females was 9% - 28% and in males 0% - 6% where as in our study number of Barr bodies in females of 9.2% - 45.7% is higher, yet in males it was almost similar.

Persistence of Sex chromatin

In the study we were able to differentiate sex with certainty up to four weeks only which is almost similar to five week duration of Whittaker et al [12]. Though with decreased accuracy they could determine up to 10 weeks which is in contrast to Seno & Ishizu [11] who gave this duration as 5 months.

Effect of Temperature and Humidity

As far as our knowledge is concerned, no other study has been done keeping in view the temperature and humidity so our results of temperature and humidity may be useful for further studies which are needed to confirm or negate these finding though in an experiment Duff et al [13], they heated the pulp chamber with thermo-couple probe. They found that the sex chromatin could be detected up to 75°C but above this temperature, it was destroyed and could not be detected.

CONCLUSIONS

1. The sex determination from human tooth pulp in cadavers was possible up to a period of four weeks.
2. Mean Percentage of Barr bodies in females was found to be 24.92% +/- 3.74% and the F-bodies to be 2.27% +/- 1.30%.
3. Mean Percentage of F-bodies in males was found to be 35.64% +/- 6.49% and Barr bodies to be 2.12% +/- 1.41%.
4. It was found that accurate diagnosis from human teeth, the number of Barr bodies in female samples should be more than 6% and number of F-bodies in males should be more than 8% up to a period of 4 weeks time since death.
5. There was a remarkable fall in the sex chromatin of pulp cells in the period of 6 – 24 hours.
6. The bacteria, dead cells, and putrefied cellular debris were a constant nuisance in the determination of sex from pulpal tissue. The post-mortem fragility of the cells poses a problem in the separation of cells from the hard pulpal tissue. Thin smears facilitate the clear visibility of the intact and visible cells.
7. A join search for the presence or absence of Barr bodies and F-bodies should be made to establish the sex from human tooth pulp tissue because in tropical countries like India where there are wide variations in temperature and humidity, the pulpal tissue undergoes putrefaction quickly. A negative result of either of sex chromatin may give a wrong diagnosis of the sex of the individual.

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