Sex Chromatin and Nuclear Sex-Specific Appendages in the Newborn

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Since Moore and Barr developed a technique to determine sex-chromatin by buccal smear in 1955[1] different types of studies have been made on this subject.

A low incidence of sex-chromatin during the first few days of life has been found by many authors. [2-10] Surveys have been made for abnormal sex-chromatin findings in large groups of patients admitted to mental hospitals and hospitals for the mentally subnormal as a screening test for further chromosomal analysis. [11-13] In other large surveys of newborns, abnormalities of the sex-chromatin have again been detected. [14-16]

It has also been shown that the polymorphonuclear leukocytes of the female have peculiar nuclear appendages, drumsticks, sessile nodules and clubs, and that the percentages of these are extremely low in males. [17 18]

Many investigations have been made to find the possible causes for the low incidence of sex-chromatin during the first few days of life. Takemi et al thought that it is may be related to the period of gestation[7], while Hsu et al and Curtis suggested that it was due to the different nature of the buccal epithelium in relation to the day-age of the baby.[2 10] Golob et al discussed the relation of sex-chromatin to estrogenic activity in newborn females.[8]

The purposes of the present study are to determine the incidence of sex-chromatin in a group of newborn and adult Turkish patients, both

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male and female; to prove or disprove the effects of the gestation period or environment on sex-chromatin levels in infants; and to find a possible correlation between sex-chromatin and nuclear sex-specific appendages of the polymorphonuclear leukocytes in different groups.

Materials and Methods

Newborns

A total of 81 newborns (41 male and 40 female) were studied in two groups. The first consisted of 31 vaginally delivered infants (16 male and 15 female) from the maternity ward of Hacettepe Hospital. Buccal and blood smear examinations after three days of life were made on only nine females and 13 males, the rest being discharged earlier.

The second group consisted of 50 newborn infants delivered by cesarean section at the Ankara Maternity Home (25 male and 25 female), from whom 49 buccal smears were examined before and after the third day. The period of gestation and birth weights of the infants in both groups were recorded.

The infants were divided into three groups similar to those used by Takemi et al; the gestational age of the full-term infants varied between 37 and 44 weeks and the premature infants were divided into two subgroups of 32 weeks' gestation and below, and over 32 weeks.[7]

The criteria for Turkish children mentioned by Doğramacı[19] were adopted, and the infants weighing 2,500 gm and under were accordingly placed in the premature group.

None of these children was under any antibiotic treatment.

Adults

Group 1: 21 healthy and physically normal women. In 15 the sex was already confirmed, as they were the mothers of children (patients attending the pediatric hematology outpatient department). The rest were unmarried with normal menstrual histories. Their ages ranged between 18 and 44 years.

Group 2: 11 pregnant women attending Hacettepe maternity outpatient department for routine check-up at or over the 32 week gestation period, aged between 19 and 44 years. All were normal, and none was under any treatment, hormonal or antibiotic.

Group 3: 20 physically normal males aged between 19 and 60 years. Twelve were proved fertile, being the fathers of children (patients attending the pediatric hematology outpatient department).

Four smears, two from each side of the cheek, were obtained from the newborn infants of both groups, one set within the first three days of life and the other mostly on the fourth or fifth days. Smears from the adult female, pregnant and male control groups were also obtained, two in each case, one from each side of the cheek. The slides were labelled to show case number, from which side of the mouth, and, in the case of infants, the first or second set of smears.

Buccal smears were taken by scraping the inside of the cheek with a clean tongue depressor, and smearing the matter gently onto a clean, dry slide which was immediately immersed in fixative consisting of three parts 95 per cent ethyl alcohol and one part glacial acetic acid. Fixation continued for at least 10 minutes and sometimes longer, until statining.

A modified aceto-orcein stain[20] was used. The slides were then laid horizontally on blotting paper, and four drops of the stain were immediately placed on them (two on each half of the slide); each one was then covered with two coverslips and the stain was left for seven to 10 minutes. At the end of the staining period, residual stain was squeezed off under two layers of blotting paper, and the cover slips were cleaned with moist gauze to remove the superflous dried stain from their top surfaces. The slides were either examined immediately under oil immersion lens or left in the deep freeze for a day or so until examination.

Careful selection was made of the areas of the slides where the cells were to be counted. Bacterial contamination was not encountered in any of the slides. Nuclei which were non-pyknotic, non-vacuolated and without coarse granules, flat or oval in shape with clear, unbroken and unwrinkled membranes were included, and only those with sex-chromatin adjacent to the nuclear membrane were considered.

One hundred such nuclei were examined for sex-chromatin from each of the slides in the adult females, pregnant women and males, and 100, one from each side of the cheek, in the case of infants. The extra two slides from either side were often needed when the first were unsatisfactory, usually due to degenerated cells, and where repetition would have been necessary.

The results of the percentage of sex-chromatin positive cells from both sides and their means were recorded separately in each case.

TABLE I

MORPHOLOGICAL CRITERIA FOR CLASSIFYING NUCLEAR EXCRESCENCES (SEX-SPECIFIC) IN NEUTROPHILIC LEUKOCYTES

Name	Size	Head	Stalk	Incidence	Comments
Drumstick	1.5 U	ovoid or spherical	Thin	1-14% of normal female neutrophils (mean 5 %)	Related to the No. of X chromosomes present
Sessile	1.5 U	ovoid or spherical	Absent	1-12 % of normal female neutrophils (Mean=5%)	related to the number of X chromosomes present
Club	1.3	Club shaped	Broad with tapering base		

Comparisons of Results of Statistical Analyses of All Groups

1. Mean sex-chromatin findings of Group 1 in the first three days of life and after three days of life;
2. Mean sex-chromatin value of Group 1 in the first three days of life and after three days with the adult normal female group;
3. Group 2 was studied similarly to Group 1;
4. Groups 1 with 2 for the mean sex-chromatin values in the first three days of life and after three days.
5. The adult normal female group with the pregnant women group.

Blood smears were obtained simultaneously with the buccal smears from all groups except that delivered by cesarean section as permission to obtain them was unavailable; these were taken on cover glasses [21] and stained with Wright's stain.

One hundred or more polymorphonuclear leukocytes were examined on each slide, and the percentage of drumsticks, sessile nodules and clubs were recorded separately.

Statistical Analysis of Blood Smears

Correlation of mean sex-chromatin, drumsticks, sessile nodules, clubs and a total of the latter three in the newborn Group 1 during the

first three days, and after three days of life, was evaluated. Similar studies were made on the blood smears of the adult female and pregnant women groups.

Statistical comparisons were made between Group 1 on the first and third days of life, and again compared to the adult normal female control group.

Results

Sex-Chromatin in Newborn Infants

Group 1: Out of 15 newborn females, delivered vaginally at Hacettepe Hospital, only nine were available for second examination after three days of life. The mean of sex-chromatin positive nuclei was 18.6 per cent during the first three days of life, and 32.9 per cent after three days, with a range of nine to 23.5.

There was a statistically significant difference in mean sex-chromatin frequency during the first three days of life and three days after birth, $P < 0.001$.

Relation of the period of gestation and birth weight to sex-chromatin:

a) Normal gestation and birth weight - no statistically significant correlation present during the first three days of life, $P > 0.05$. These findings were similar after three days of life.

b) High gestation, low birth weight: (only one case) the mean sex-chromatin during the first three days of life was 20.5 per cent and 30.0 per cent after three days.

c) Low gestation low birth weight: (only two cases in the first three days of life). In one the mean sex-chromatin was 23.5 per cent and in the other 19.0 per cent.

Compared to the adult normal female group, there was a statistically significant difference in mean sex-chromatin during the first three days of life, $P < 0.001$, but not after three days of life, $P > 0.05$.

All the male newborns were negative for sex-chromatin in both periods.

Group 2: (Delivered by cesarean section at Ankara Maternity Home.)

The mean incidence of sex-chromatin studies in 25 newborn females during the first three days of life was 13.7 per cent, and after three days

in 24 infants, 25.3 per cent. The range of the mean was eight to 19 per cent during the first three days, and 19.5-42 per cent after three days.

A statistically significant difference was observed in the mean sex-chromatin during the first three days and three days after birth, $P < 0.001$

Relation of the period of gestation and birth weight to sex chromatin:

a) Normal gestation and birth weight - no significant correlation during the first three days of life - $P > 0.05$. The findings were similar after three days of life.

b) High gestation, low birth weight - no cases available.

c) Low gestation, low birth weight - no cases available.

There was a statistically significant difference between the mean sex-chromatin in the newborn females delivered by cesarean section during the first three days of life and that of the adult normal female group, $P < 0.001$, but not after three days of life, $P > 0.05$.

In Cases 12 and 33 the buccal smear showed one per cent of double sex-chromatin on the fifth day after birth on both sides of the cheek, which may have been mosaicism. Smears were repeated eight days after birth, and in Case 12 double sex- chromatin was two per cent, and in Case 33 one per cent. Dermatoglyphic studies of Case 12 showed ulnar-type loops on all fingers of the hands. Neither patient was brought back for chromosomal analysis in the Genetic Clinic of Hacettepe Hospital after discharge from the Maternity Home as requested.

Case 9 expired before the fourth day. All the male newborns in the group were negative for sex-chromatin.

No statistically significant difference was observed in the mean sex-chromatin frequency between the first and second groups during the first three days and after three days of life, $P > 0.05$.

Sex Chromatin in Adults

Females: The mean value was 29.8 per cent with a range of 19-40 per cent.

Pregnant Women: The mean value was 28.7 per cent with a range of 23–39 per cent. There was no statistical difference in mean sex-chromatin findings between the adult normal females and the pregnant women $P > 0.05$.

Males: All were negative for sex-chromatin.

In none of the groups, infant or adult, was the difference in the statistical findings between the sex-chromatin of the right and left sides of the cheek significant.

Mean percentages of sex specific appendages of polymorphonuclear leukocytes in all the groups studies are given in Table II. The percentages of drumsticks, sessile nodules, clubs and their total were greater in the newborn females (Group 1) during and after the first three days of life than in the adult normal female control group.

TABLE II

MEAN PERCENTAGES OF SEX SPECIFIC APPENDAGES OF POLYMORPHONUCLEAR LEUKOCYTES

Group	Drumstick	Sessile Nodules	Club	Total of Drumsticks Sessiles and Clubs
Newborns (Group 1) First 3 days of life	4.6	2.5	1.4	8.5
After 3 days of life	4.7	2.3	2.9	9.90
Adult normal female control group	1.0	1.5	0.5	3.0
Pregnant women	1.46	2.15	0.3	3.91

The mean values of sex-specific appendages of polymorphonuclear leukocytes in the female control group and those of newborn females (Group 1) during and after three days of life were compared by Tukey's methods as shown in Table III.

Statistically significant differences were found in the mean drumstick frequencies of Group 1 during the two periods of life compared to the female control group, but there were none between the first three days of life and after three days.

There was a statistically significant difference in the mean frequency of sessile nodules between Group 1 in both periods of life and the adult female control group, and also between infants in the first three days of life and after three days.

The results of the mean frequency of clubs were similar to those of sessile nodules.

TABLE III

Differences between means values	D. Values	
Drumsticks		
I - II = 3.97	>0.495)	statistically
I - III = 4.328	>0.495)	significant
II - III = 0.214	>0.495)	not statistically significant
Sessiles		
I - II = 1.339	>0.413)	
I - III = 1.991	>0.413)	statistically significant
II - III = 0.429	>0.413)	
Clubs		
I - II = 0.825	>0.252)	
I - III = 2.232	>0.252)	statistically significant
II - III = 1.357	>0.252)	
Total of Drumsticks, Sessiles and Clubs		
I - II = 5.892	>2.499)	
I - III = 6.607	>2.499)	statistically significant
II - III = 0.715	<2.499)	not statistically significant

I = Mean values of drumsticks, sessiles and clubs in adult females

II = Mean values of drumsticks, sessiles and clubs in newborn females (first three days).

III = Mean values of drumsticks, sessiles and clubs in newborn females (after three days).

The total percentage of drumsticks, sessile nodules and clubs showed a statistically significant difference in both periods of newborns in Group 1 compared to the adult female group, but not compared to the infants in Group 2.

A similar comparison was made between the adult female control group and the pregnant women. No statistically significant differences were found between either the results or the total number of drumsticks, sessile nodules and clubs, $P > 0.05$.

The males of all groups were negative for sex-specific appendages.

Correlations between mean sex-chromatin, and the sex-specific appendages of polymorphonuclear leukocytes in all groups are presented in Table IV.

TABLE IV

CORRELATION BETWEEN THE MEAN SEX-CHROMATIN AND THE SEX-SPECIFIC APPENDAGES OF POLYMORPHONUCLEAR LEUKOCYTES

Group	Drumsticks	Sessile Nodules	Clubs	Drumsticks, Sessile nodules and Clubs
1 (First 3 days of life)	No statistically significant correlation $P > 0.05$	Statistically significant and positive correlation $P < 0.05$	Statistically significant and positive correlation $P < 0.02$	No statistically significant correlation $P > 0.05$.
(After 3 days of life)	No statistically significant correlation $P > 0.05$	Statistically significant and negative correlation $P < 0.02$	Statistically significant and negative correlation $P < 0.001$	Statistically significant and negative correlation $P < 0.05$
Adult Female Group	No statistically significant correlation $P > 0.05$	No statistically significant correlation $P > 0.05$	No statistically significant correlation $P > 0.05$	No statistically significant correlation, $P > 0.05$
Pregnant Women	No statistically significant correlation $P > 0.05$	No statistically significant correlation $P > 0.05$	No statistically significant correlation $P > 0.05$	No statistically significant correlation > 0.05

Discussion

Sex Chromatin

It is well known that sex-chromatin frequency in newborn females is low at at birth and rises thereafter[3-7]. Our study, too, confirmed that the incidence was lower during the first three days of life than afterwards in vaginally delivered females. Statistical analyses also revealed significant differences in these periods, as has previously been reported by other authors.[2 7]

Similar findings were noted in the newborn females delivered by cesarean section; a low incidence of sex-chromatin in newborn females thus delivered has also been reported previously.[9]

In this study the mean sex-chromatin of newborn females during the first three days of life compared to the adult female control group was low in both groups, but no significant difference was observed after three days of life, or between the groups in both periods. That sex-chromatin frequency almost reaches adult levels after three days of life has already been reported.[3] These findings lead one to believe that there are factors responsible for altering the incidence of sex-chromatin during delivery, the gestation period or the first few days of life.

Many investigations have been made to find possible causes for low sex-chromatin at birth in newborn females. It has been suggested that a common, possibly humoral factor is responsible in both the baby and the mother, because there is a decrease in sex-chromatin in pregnant women 24 hours prior to delivery, and an increase by the second postpartum day.[3]

It was also found that sex-chromatin did not fluctuate in the mothers of newborn females delivered by cesarean section as it did in those of vaginally delivered females, though the results were similar in both groups of newborns. This suggests that the mode of delivery or factors during labor are not related to the low incidence of sex-chromatin, but to the environmental alterations of the buccal cells from amniotic fluid to atmospheric exposure.[9]

On the other hand, Takemi et al showed that there is no transient supression of sex-chromatin in low birth weight, low gestation period groups, but that there is in low birth weight, high gestation and normal birth weight, normal gestation period groups. (All these infants were equally exposed to the atmosphere). They concluded that transient sex-chromatin suppression was related to a physiological processs that develops during the last eight weeks of gestation.[7]

We could make no such confirmation as the number of infants in the high gestation period, low birth weight and low gestation period, low birth weight groups was insufficient. However, no statistically significant correlation was present in the group with a normal gestation period, normal birth weight and mean sex-chromatin during and after three days of life. Similar results have also been reported previously.[5-7]

The effects of hormones have been studied by various authors. One found no correlation between sex-chromatin and the menstrual cycle,[22] while another reported a decrease in sex-chromatin during days nine to 12 of the menstrual cycle.[23] These findings are contradictory, and remain unconfirmed. Sex-chromatin frequency in relation to estrogenic activity has been studied in newborns in their buccal and vaginal epithelial cells, and it has been suggested that estrogens could be the causal factor.[8]

We found no statistically significant difference in the mean sex-chromatin of the adult normal female control group and that of the pregnant women. Estrogen levels are very high during pregnancy,[24] but there was no low frequency of sex chromatin during this period compared to newborn females.[2] Our results were similar, suggesting that hormones could not possibly cause low sex-chromatin during the first few days of life.[2]

Our findings also support those of Hsu et al[10] and Curtis[2] that the buccal epithelium of newborn infants may have more degenerative cells which slough off during the first few days of life through normal physiological processes; they too discarded more slides of newborn females than adult females due to degenerated cells. This degeneration may start in the last eight weeks of pregnancy, as no suppression of sex-chromatin was observed in the newborn females with low gestation periods.

To confirm the relation of the gestation period, sex-chromatin may be determined in fetuses at different week-ages. Whether or not the degenerative changes are only in the buccal mucosa should also be confirmed by studying other tissues of the same infant or fetus simultaneously.

Sex-specific Appendages of Polymorphonuclear Leukocytes

These appendages were studied in the newborns, normal adult females and pregnant women to find their frequencies and correlations to sex-chromatin. The results include all the sex-specific appendages. i.e. drumsticks, sessile nodules and clubs, recorded separately as well as in total. The percentages of all these were found to be greater in the newborn females than in the normal adult females (see Table II).

It has been reported that the percentage of drumsticks is higher in females in early childhood than in normal adult females due to hormonal influences.[25 26] Our study confirms this increased incidence.

Sex-chromatin frequency is low in the newborn period, as is seen from this and previous studies,[3-5] and after the administration of prednisone.[28] However, the frequency of drumsticks is higher at birth[25 26] and after the administration of adrenocorticotrophin (ACTH) and insulin.[29] This suggests that there are some effects from hormonal changes but with different results, and that both sex-chromatin and drumsticks are related to sex.[17]

We found no significant difference in the frequency of sex-specific appendages between the normal female controls and the pregnant women. As hormone levels, especially estrogens,[24] are much increased during pregnancy, an increase in drumsticks and other sex-specific appendages of the polymorphonuclear leukocytes should have been present in the pregnant women.

The incidence of drumsticks depends to some extent on the degree of segmentation of the nuclei of the polymorphonuclear leukocytes; it is high with high segment counts,[30 31] and often low with low segment counts.[32] It has also been suggested that drumstick counts are decreased in pregnancy due to a shift to the left.[33]

It is not clear if the factors affecting buccal smear and polymorphonuclear leukocyte findings are different or if they have the opposite effects.

No statistically significant correlation was observed between the mean sex-chromatin and the drumsticks in Group I (both periods), the adult females or the pregnant women. There was, however, between the sex-chromatin and the sessile nodules of the newborns during the first three days, but it was statistically significant and negative after three days. Similar results were present for the clubs. Such correlations were absent in the adult normal females and the pregnant women.

The causes of the correlation during the first three days of life, which did not exist in the adult groups, and the sudden changes after three days are not understood. Longitudinal research on the newborn may be required to study the subject further and find at which stage the changes begin, and at what age the percentages become similar to those of adult females.

There was no statistically significant correlation between sex-chromatin and the total of sex-specific appendages of the neutrophils in the

newborn females during the first three days of life and the normal adults and pregnant women, but there was after three days of life. It is difficult to find an explanation for this difference.

Conclusions

It is concluded that the incidence of sex-chromatin in the buccal smear of newborn females delivered both vaginally and by cesarean section is low during the first days of life compared to later, and to adult females. The results after three days are almost similar to those of adult females. The frequency of sex-chromatin in the adult females and pregnant women was also identical.

The low frequency of sex-chromatin in newborn females appears to be due to local changes in the buccal epithelium at birth, and to the increase caused by physiological changes thereafter. On the other hand, the incidence of all sex-specific appendages of the polymorphonuclear leukocytes is higher during and after three days of life compared to the normal adult female control group.

No statistically significant correlation was found between sex-chromatin and the total sex-specific appendanges of the polymorphonuclear leukocytes in any group, except that there was a statistically significant and negative correlation in the newborns after three days of life, the causes of which could not be found.

Summary

A total of 81 newborns (41 male and 40 female) were studied for sex-chromatin frequencies in the buccal mucosa during the first three days of life and after three days.

Fifteen female and 16 male newborns delivered vaginally (Group 1) were also studied for sex-specific appendages of the polymorphonuclear leukocytes. The remaining 25 female and 25 male newborns delivered by cesarean section (Group 2) were studied for sex-chromatin only.

The incidence of mean sex-chromatin was low in the newborn females during the first three days of life compared to that after three days in both groups, and almost similar to the adult normal female control group. The incidence was identical in both the infant groups in both periods of life. It appears that the cause of supression of sex-chromatin at birth is due to local changes in the buccal epithelium, and is related to normal physiology of the buccal mucosa.

The incidence of mean sex-chromatin was identical in the normal female control group and the pregnant women.

No statistically significant correlation was found between mean sex-chromatin and sex-specific appendages of the polymorphonuclear leukocytes in any group studied, except that in the newborns a statistically significant and positive correlation between sex-chromatin and sessile nodules was present during the first three days of life. This also applied to the clubs. A statistically significant and negative correlation of the sessile nodules and clubs and between the sex-chromatin and the total of drumsticks, sessile nodules and clubs was present after three days of life, but not during the first three days. The reason for this change is not understood.

Longitudinal studies of a large group of infants for sex-specific appendages are suggested, as are sex-chromatin studies of fetuses at different gestation periods, and of other tissues in addition to the buccal smear.

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