Observations on Study of Sex-Chromatin in Cases of Developmental Anomalies of Urogenital Tract

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Within the last fifteen years, several discoveries have altered the so called 'established facts' in the interesting field of sexual anomalies. Many attempts have been made to disclose the mystery of biological configurations controlling the emergence of inherited characters. Recent research definitely draws our attention towards the gene which is responsible in the biological entity to to modulate the adult characters (Bently Glass, 1957; Allison, 1959). It is now almost established that the structural and functional aspects of individual cells are related to genetically controlled developmental processes. The brilliant discovery of Barr and Bertram of Western Ontario University discloses an important structure inside the nucleus of somatic cells, which ramifies the biological specificity of male and female sex. They are called sex-chromatin, or the Barr bodies. This discovery of sex difference has stimulated many outstanding investigations on several perplexing sex syndromes.

Since the discovery of chromosomes, it was almost taken for granted that the number of chromosomes in the human species was '48'. J.H. Tjio and Albert Levan (1956) made this spectacular discovery of finding '46' chromosomes in the cultured cells of the human embryo. A. Jacob and J. A. Strong discovered '47' chromosomes in the cells of patients of Kline-Felters syndrome. In Turners syndrome, it is '45' in number, and in Mongolisms, usually a trisomic group of 21-22 is found.

This normal complement in man, i.e. 22 paired autosomes associated with an X and a Y sex-chromosome is now generally accepted. The basic difference in gender is seen in the sex-chromosomal pattern, i.e. XX in female, and XY in male.

There is definite confirmation that an ovum harbours two X chromosomes and a spermatozoon carries an X chromosome and a smaller Y chromosome (Cabot, 1946). Genetically, sex is determined at the very moment of fertilization of an ovum by a spermatozoon carrying an X or Y chromosome. Combination of XX chromosomes develops into a female and XY chromosomes develop into a male. With the advent of new concept in cellular, structure chromosomal configurations become most helpful in distinguishing and treating sex-anomalies. In doubtful cases of inter-sex, (Hermaphroditism; Psuedo-hermaphroditism, etc.) a clinician can get helpful guidance by microscopic examination of somatic cells of the patient for the presence of sex-chromatin in them.

Sex-chromatin :

Innumerable studies have substantiated

the validity of sex-chromatin in human beings. It is thought that sex-chromatin is derived from sex-chromosomes which tend to be stabilized into a compact mass, and stain heavily with basic dyes (Allen, Danforth, and Doisy, 1939). Whereas, autosomes or Somatic chromosomes are diffused and stained lightly. It is therefore considered that the apposition of two X-chromosomes produce the typical deep pyknotic appearance. The mass is sufficiently large enough to be readily visible under oil emersion lens of the microscope. The small size of the Y-chromosome is not visible in the cells of males (Ford, 1960).

The sex-chromatin unit measures about 1μ in diameter (Kieffer, 1957). It is plano-convex, contains nucleic acid, mainly of the desoxyribose type (Shettles, 1956) and is usually found in the inner surface of the nuclear cell membrane.

The percentage of Sex-chromatin bodies in the female cell ranges from 10-95, while the appearance of such nuclear masses is only a few or totally absent in the males.

The sex-chromatin masses are localised in several varieties of tissue cells. It has been ovserved in nerve cells (Barr and Bertram, 1949, and Hay and Moore, 1961), Polymorphonuclear neutrophill leucocytes (Davidson and Smith, 1954), Kumaron and Iya, 1964), cells of buccal mucosa (Moore and Barr, 1955), cells of amniotic and allantoidic fluids (Sen et al, 1955; Neimann-Soren Sen, 1957), vaginal exfoliate cells (Carpentier et al, 1955), cells from urinary sediments (Cantro et al, (1957), in the embryonic tissues of females, (Marberger and Nelson, 1965), Dental cells (Castras et al, 1959), and from cells of hair and skin buried for sometime (Moore and Barra, 1953).

The cells commonly used for rapid sex determination are those from the skin, blood, mucous membrane, and amniotic fluid. Scrapping from the epidermis and oral mucosa or smears from the vagina may be prepared and examined. It is customary to observe at least 500-1000 cells before a diagnosis is made.

Sex differences are also seen in the circulation neutrophills which appear as accessory nuclear lobes or 'Drum Stick' appendages. The 'Drum Stick' is characteristic to matured neutrophills, only rarely seen in unsegmented forms and never observed in the precursor cells. A patient with an acute, severe infection display, a toxic shift to the left, may fail to show the characteristic 'Drum Stick'. A blood smear from a female presenting a shift to the right will be taken with the diagnostic chromatin pattern.

Davidson and Smith (1954) describe the 'Drum Stick' projections from one of the lobes of polymorphonuclear neutrophill leucocytes of the human blood smear. This is an ovoid body of dense chromatin, 1.5μ in diameter, which is attached to the lobe of the nucleus by a slender filament (Riis, 1956 and Briggs, 1958). (Kosenow and Scupine, (1956) described appendages of similar size but without a stalk. These cessile modules are equally sex-specific.

The main aim of the present study is to carry on estimation of sex-chromatin in

some common congenital genito-urinary abnormalities and to assess whether the congenital abnormalities are genetically predetermined. The importance of sex-chromatin mass is demonstrated in differentiating – hermaphrodites, Psuedo-hermaphrodites, or individuals who are phenotypically deviated from normal sextype. (Bergmann, 1961). It would be difficult to obtain a figure regarding the number of so called Psuedohermaphrodites. Now phenotypes are being described currently as investigating procedures to become more diagnostic. As mentioned previously, some hermaphrodites have an XO (Turner syndrome), or an XXX (Kline-Felter syndrome), Chromosomal pattern, (Turner, 1938. Kline-felter, Riefenatein, and Albright, 1942). So, in those above cases, by estimating the percentage of sex-chromatin and Drum Stick, it can be predicted whether the affected person is more towards male or female character genetically.

Clinical application of sex-chromatin by counting the number in a special group of cells is almost entirely confined to the human species. The value of sex-chromatin determination appears to be of prime importance in patients with infertility or failure of development of secondary sexual character.

This new dimension in the appraisal of patients sex has its own role in the study of Inter-sex. Nelson, (1955) and Barra, (1956) have investigated testicular disorders and observed a female sex-chromatin pattern. Crumback et al, (1957) investigated 65 phenotipic males with a variety of testicular disorders. Patients with testicular fibrosis or germinal aplasia were found to have female sex-chromatin pattern. Detection of the patient's sex-chromatin has now become an indispensable clinical procedure for the study of human gonoidal dysgenesis. Its value is well recognised in planning the endocrinological rehabilitation of the sexually disabled.

Application of Nuclear-sexing :

1. Inter-sex—This is by far the most important application amongst the various kinds of Inter-sexes with its widest range of deviations (Social, Psychological, Anatomical, Endocrinal, or chromosomal) from the strict norms of male to female.

2. Antenatal Sexing—From the cells which float in the amniotic fluid. It is just possible to determine the sex of the child before its birth even in the third month of intra-uterine life.

3. Placental Anatomy-when the foetus is male, the sex of the contained nucleai may be used as a criterion, to distinguish maternal from the foetal tissue in the placenta.

4. Grafts-Nuclear sexing would seem to be a very obvious means of the following fate of graft transferred from one sex to another. This makes possible important contributions to the study of homotransplant problems.

5. Tumours-The majority of the tumours are recognisable of the same nuclear sex as the patient (Moore and Barr, 1965). With more actively growing tumours, anomalies such as doubled or absent sex-chromatin may occur (Aitken, 1958,

and Travarcs, 1957). Travarcs suggested that anomalies are particularly common in a group of basal cell carcinoma. More significant anomalies have been noted in two special groups of tumours :

1. Chorion epitheliomata
2. Teratomata.

Clinical application of Barr's technique is almost entirely confined to the human species. The value of sex-chromatin determination appears to be of great significance in patients with infertility, or with failure of development of secondary sex-characters. (Nelson, (1955), Bradbury et al, (1956), and others have investigated testicular disorders and observed a female sex-chromatin pattern. Patients with testicular fibrosis and germinal aplasia were found to have female sex-chromatin pattern.

In the present study an observation of the relationship between the chromosomal sex and the apparant sex of the individual has been made. This includes the study of the different factors said to be responsible for the genetic abberation of the sexes. Effects of age; sex; heredity; environment; infection, and other factors said to influence urogenital developmental anomalies are to be analysed. (The percentage of sex chromatin varies in sex and in age to age.)

The object of the present study is to find out the relationship of sex-chromatin, i.e, the genetical sex with different phases of life and specially with urogenital developmental anomalies.

Detailed case histories were taken of the patients at the department of Plastic Surgery of Patna Medical College Hospital. Special emphasis was given to :

1. Family history.
2. Developmental anomalies, if any. (Spina bifida, accessory auricle, cup-shaped deformity of ear, Mandibular agnesia, cleft lip, cleft palate, etc.)
3. Urogenital developmental anomalies.
4. Detailed local examination of the external genitalia.
5. Examination of the other systems, such as the respiratory system, C.N. system, etc.

After examining the patient on the above lines, the following was collected from the patients for the estimation of the percentage of sex-chromatin and Drum Sticks :

1. Buccal mucosa—Scraped out from the oral cavity by the blunt edge of a horn spatula.
2. Peripheral blood film—Drawn on a slide after pricking the finger tip of the patient.

The oral mucosa was smeared on slides and stained with Schiff's reagent.

Blood films were drawn on slides. They were stained by Lushmans reagent. Thus 500 cells of the oral mucosa, and 500 neutrophils from the blood smear were counted on each case to detect the percentage of "Sex-Chromatin" and "Drum-Stick" (Fig. 1, f & g).

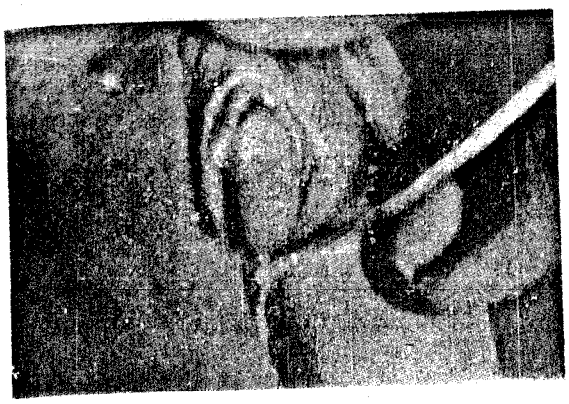

Fig. 1 (a) Shows rudimentary penis, perenial urethral orifice pointed by a pointer. Scrotale Skin is visible, in the two sides of the root of the penis.

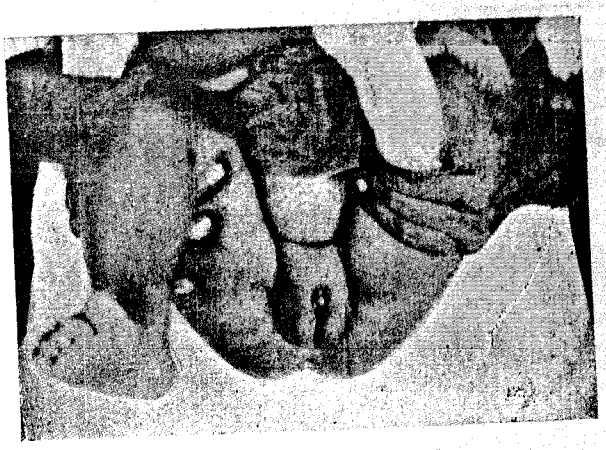

Fig. 1 (b) Shows the external ganitalia of a 7 months old patient, where a rudimentary and bifid scrotum is visible. Genetically the patient is female.

Fig. 1 (c) Illustrating a penile hypospadias, the external urethral orifice at the root of the penis.

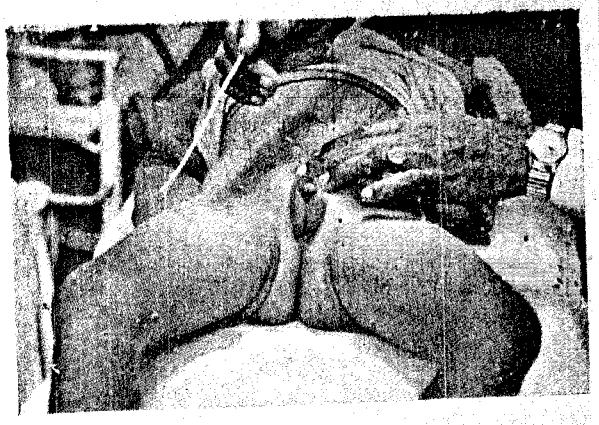

Fig. 1 (d) – Shows a glandular hypospadias

Fig. 1 (e)—Shows a penile hypospadias.

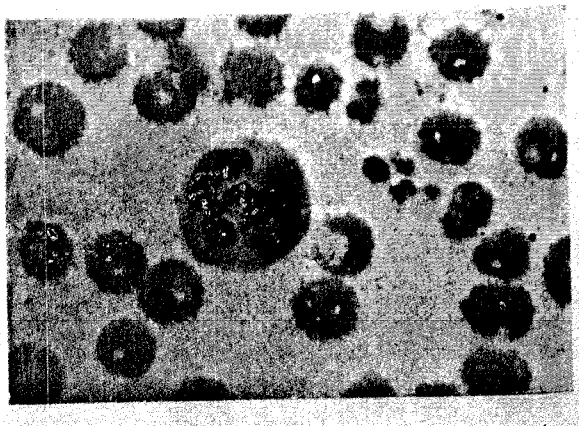

Fig. 1 (f)—Shows a Drum-stick present inside the polymorphonuclear neutrophil leucocyte, connected with the nuclear mass with a filament.

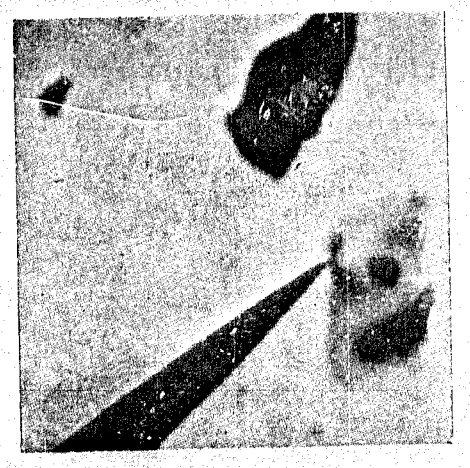

Fig 1 (g)—Pointing the sex-chromatine body, present inside the nucleus, near the respective nuclear membrane of the cells of stratified squamous epithelium of the oral mucosa.

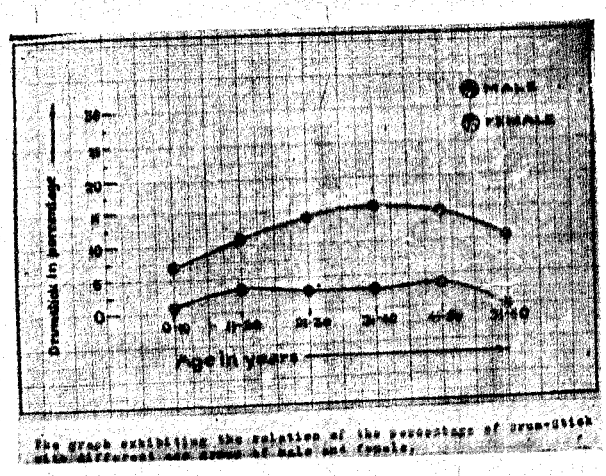

Fig 2

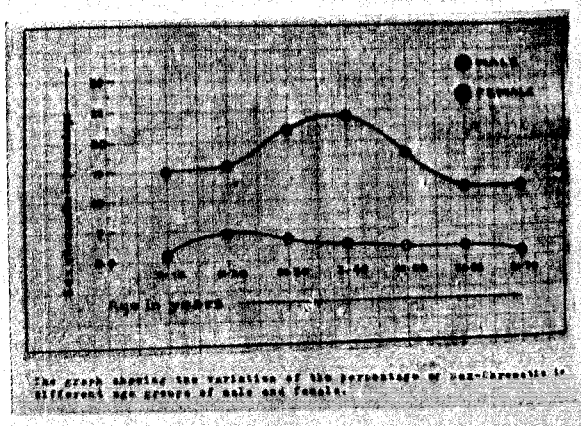

Fig. 3

Observations

Findings of this experimental work are given below in tabulated form :

A. Observations on cases without Urogenital Developmental Anomalies, i.e., otherwise normal individuals. (Number of cases studied-100).

Table I

Shows the total number of cases studied without Urogenital Developmental Anomalies in term male and female amongst different age groups.

Total	Male	Female
100	50	50

Table II

Depicts the respective percentage of male and female in different age groups.

Age group in years	Total No. of cases	Male	Percentage	Female	Percentage
8—17	19	9	47	10	53
18—27	28	17	60	11	40
28—37	21	13	62	8	38
38—47	21	7	33	14	67
48—57	5	2	40	3	60
58—67	3	1	33	2	67
68—70	3	1	33	2	67

Table III

Shows the number of male and female with their percentage found within different percentage range of the Drum Stick (Fig. 2).

Drum Sticks in percentage	Total No. of cases	Male	Percentage	Female	Percentage
0—5	54	49	91	5	9
6—10	23	1	4	22	96
11—15	6	0	0	6	100
16—20	13	0	0	13	100
21—26	4	0	0	4	100

Table IV

Table shows the number of male and female with their percentage found within different percentage range of the sex-chromatin (Fig. 3).

Sex-chromatin in percentage	Total No. of cases	Male	Percentage	Female	Percentage
0— 5	48	48	100	0	0
6—10	13	2	15	11	85
11—15	10	0	0	12	100
16—20	18	0	0	18	100
21—26	6	0	0	6	100
27—32	4	0	0	4	100
33—40	1	0	0	1	100

Table V

Shows the relation between Drum Stick in percentage and the Sex-Chromatin in percentage.

Drum Sticks in percentage	Sex-Chromatin in percentage							
	0—5	16—10	11— 15	16—20	21—25	26 30	31—35	36—40
0—5	47	5	3	0	0	0	0	0
6—10	0	8	6	7	1	1	0	0
11—15	0	0	0	6	0	0	0	0
16—20	0	1	1	3	5	0	0	1
21—25	0	1	0	1	0	1	0	0
26—30	0	0	0	0	0	1	0	0

Table VI

Narrates the number and the percentage of the cases of pregnant mothers ultimately delivered male and female child.

Total No. of mothers	No. of pregnant mothers ultimately delivered male child	Percentage of Sex-Chromatin	No. of mothers carrying and ultimately delivered female child.	Percentage
6	2	33%	4	67%

Table VII

Table narrates the number of pregnant mothers ultimately delivered male and female children in different percentage groups of sex-chromatin.

Mother delivered	Sex-chromatin in percentage			
	0—10	11—20	21—30	31—40
male child	0	0	2	2
female child.	0	0	2	2

Table VIII

Shows the number of pregnant mothers ultimately delivered male and female children in different percentage groups of Drum Stick.

Mother delivered	Drum Sticks in percentage		
	0—10	11—20	21—26
male child.	0	2	0
female child.	1	2	1

Table IX

Illustrates the maximum and minimum percentage of Sex-Chromatin and their mean values in different age groups of female.

Age in Years	Percentage of Sex-Chromatin		
	Maximum	Minimum	Mean
0—10	20	10	15.0
11—20	25	6	15.5
21—30	32	12	22.0
31—40	40	8	24.0
41—50	30	6	18.0
51—60	16	8	12.0
61—70	15	9	12.0

Table X

Table shows the maximum and minimum percentage of sex-chromatin and their mean in different age groups of male.

Age in Years	Percentage of Sex-Chromatin		
	Maximum	Minimum	Mean
0—10	1	1	1.0
11—20	8	1	4.5
21—30	6	1	3.5
31—40	4	1	2.5
41—50	3	1	2.0
51—60	4	1	2.5
61—70	3	0	0.0

Table XI

Shows the maximum and minimum percentage of Drum Stick with their mean in different age groups of female.

Age in Years	Percentage of Drum Sticks		
	Maximum	Minimum	Mean
0—10	8	6	7.0
11—20	16	5	10.5
21—30	20	8	14.0
31—40	26	5	15.5
41—50	25	4	14.5
51—60	15	6	10.5
61—70	23	4	13.5

Table XII

Shows the maximum and minimum percentage of Drum Sticks with their mean in different age groups of male.

Age in years	Percentage of Drum Stick		
	Maximum	Minimum	Mean
0—10	1	1	1.0
11—20	6	1	3.5
21—30	5	1	3.0
31—40	5	1	3.0
41—50	5	2	3.5
51—60	0	0	0.0
61—70	1	2	1.0

Observations of cases with Urogenital Developmental Anomalies (Number of cases studied—51).

Table I

Shows the total number of cases studied with Urogenital Developmental Anomalies in term of male and female.

Total	Male	Female
51	44	7

Table II

Depicts the respective percentage of male and female in different age groups.

Age group in years	Total No. of cases	Male	Percentage	Female	Percentage
0–5	24	23	95.9	1	4.1
6–10	13	12	92.4	1	7.6
11—15	7	7	100.0	0	0.0
16—20	4	0	0.0	4	100.0
21–25	3	2	66.7	1	33.3

Table III

Shows the number of male and female with their percentage found within different percentage range of the sex-chromatin.

Sex-Chromatin in percentage	Total No. of cases	Male	Percentage	Female	Percentage
0—2	39	37	94.9	2	5.1
3—4	5	4	80.0	1	20.0
5–6	5	3	60.0	2	40.0
7—8	1	0	0.0	1	100.0
9—10	1	0	0.0	1	100.0

Table IV

Shows the number of male and female with their percentage found within different percentage range of the Drum Stick.

Drum Stick in percentage	Total No. of cases	Male	Percentage	Female	Percentage
0—2	34	32	94.32		5.7
3—4	12	9	75.0	3	25.0
5—6	3	3	100.0	0	0.0
7—8	1	0	0.0	1	100.0
9—10	1	0	0.0	1	100.0

Table V

Shows the relation of Drum Stick in percentage with the Sex Chromatin in percentage.

Drum Sticks in percentage	Sex-chromatin in percentage				
	0—2	2—4	5—6	7—8	9—10
0—2	32	2	0	0	0
3—4	5	2	5	0	0
5—6	2	1	0	0	0
7—8	0	0	0	0	1
9—10	0	0	0	1	1

Conclusions

1. Percentage of sex-chromatin and Drum Stick, specially in females varies from age to age. It starts rising with the appearance of secondary sexual character, and goes down after monopause.
2. Percentage of sex-chromatin and Drum Stick in males remains more or less constant in all age groups.
3. Percentage of sex-chromatin and Drum Stick are higher in pregnant females than non-pregnant females.
4. Percentage of sex-chromatin is still higher in females carrying female child than females carrying male child.
5. Percentage of sex-chromatin and Drum Stick of psuedo-hermaphrodites, carrying both ovarian and testicular tissue, with a male type of external genitalia are higher than that in normal male.
6. Extrophy of bladder, hypospadias and ,epispadias cannot influence the percentage of sex-chromatin and Drum Stick in any way.
7. In females percentage of sex-chromatin and Drum Stick varies usually from 6 to 40%. But in males, they are from 0 to 6%.
8. *These present series of observations project light on the possible relationship between sex-chromatin, Drum Stick and the oestrogen (i e. female sex-hormone) level in the blood.*

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