INTRODUCTION AND REVIEW OF LITERATURE

The sex difference in mammalian interphase cells was first described by Barr and Bertram (1949) who discovered a darkly - stained body in the nuclei of nerve cells only belonging to female cats and not in the male cells . This body was intimately associated with the nucleolus and appeared in mature nerve cells as a small satellite stainable with basic dyes as cresyl - violet ,thionin and haematoxylin . Accordingly , the authors called it the nucleolar satellite . At first , the authors suggested that the nucleolar satellite might be derived from that chromosomal portion known as nucleolar organiser

However, Barr (1951) found that , in neuroglial cells of female cats , the dark body appeared against the inner aspect of the nuclear membrane and was not necessarily associated with the nucleolus . So , the term nucleolar satellite became inappropriate , and the term sex chromatin or Barr body was used instead .

Later on, the sex chromatin was demonstrated in various somatic cells of female cats by (Graham and Barr , 1952) . However , they failed to elucidate the morphological differentiation in liver , renal convoluted tubules and pancreatic acini cells. Still later , it was demonstrated by (Moore and Barr, 1957) in human female nerve cells .

Moore , Graham and Barr (1953) were the first to show the chromosomal sex of normal , as well as , hermaphrodite humans from preparations made of skin biopsies using the cresyl - violet method . Moreover , Marberger , Boccabella , and Nelson(1955) showed that the sex chromatin can be demonstrated in buccal mucosa cells obtained by scraping the inside of the cheek .

Many years later , Schmid(1967) demonstrated the sex chromatin also in hair roots of human females . Most recently , Amador , Stolorow and Tahir (1984) had determined the difference between male hairs and female hairs by sex chromatin staining within the nuclei of epithelial cells in hair root sheaths .

Davidson and Smith (1954) , in the same connection, discovered a sex chromatin appearing as a drum stick structure , in few percentage of polymorphonuclear leucocytes of normal females , however , absent from the cells of normal males .

Hunter and Lennox (1954) studied teratoma and benign tumours from male and female patients and found that sex chromatin was also present only in female cells .

Mukherjee , Gertrude , Moser and Nitousky (1972) showed the fluorescent characteristics of X and Y chromatin of human interphase nuclei stained with quinacrine mustard in fibroblast - like cell cultures derived from several different tissues . The X - chromatin in the interphase nuclei of female cells could be identified unambigously on the basis of its greater size and lesser fluorescence intensity per unit area when compared with the size and fluorescence intensity of the Y - chromatin in male cells . In addition , further aids to X - chromatin identification were based on its characteristic shape

would always be inactivated in the descendants of each cell . In this concept , Ohno and Makino(1961), Morishima and Taylor (1962) , Grumbach , Morishima , and Taylor (1963) , and Rowley , Muldal , Gilbert ,Lajtha , Lindsten , Fraccaro and Kaisjer (1963) found a lot of evidence which supported Lyon's postulation. They found that the number of Barr bodies in the cell was always one less than the total number of X-chromosomes . Also , the number of late labelling chromosomes was equal to the number of sex - chromatin masses , and the late labeller was neither present in normal male metaphase nor in XO females (Turner's syndrome) . The authors thus suggested that the late labelling X chromosome might represent the Barr body and they concluded that the origin of sex chromatin was from a single X - chromosome . In addition , Beutler ; Yeh and Fairbanks (1962) and Davidson ; Nitowsky and Child (1963) agreed with Lyon's hypothesis when they used glucose - 6 - phosphate dehydrogenase (G- 6-PD) as a marker for the sex linked genes . They postulated that if both X - chromosomes were active , homozygous females would have had twice as much G - 6 - PD

activity in their erythrocytes as did normal hemizygous males . Virtually , they added , G - 6 -PD activity was the same in both males and females

Based on the above data , Russel (1964) tried to provoke a question concerning the metabolic error found in female patients with XO karyotypes (Turner's syndrome) and in male patients with XXY karyotypes (Klinefelter's syndrome) despite the inactivity of the missing and the extra Xs in the two syndromes respectively . Accordingly , the author suggested , from studies on the mouse, that a portion of the inactivated X - chromosome might not be inactivated . The inactive X - chromosome might contain active loci to be presented in duplicate , if normal development and function was to take place .

Further more , Ferguson (1965) had proposed that there are loci on the Y chromosome homologus with certain loci on the X - chromosome and thus , the normal XX female and normal XY male have these loci in duplicate . On the other hand , the XO Turner females would be deficient in these loci

and the XXY Klinefelter males would have these loci in triplicate . The authors also noticed that the absence of the short arm of the X -chromosome would seem to be responsible for the body features associated with the XO Turner's syndrome . In this context , Rothawell (1977) has supposed that it is the most of the long arm which becomes inactive and most or all of the short arm remains active .

It has been generally accepted that the frequency of sex chromatin varies in accordance with the source of the studied material , and the nature of material ; i.e. , wheather it is buccal or vaginal smear , sectioned material or whole mounts of foetal or mesentric membrane..... etc. For example , the highest frequency of sex - chromatin positive cells is found in amniotic cells in which about 95% are sex - chromatin positive . In cells of buccal mucosa , the incidence of Barr bodies is ranging from 20 to 80 % in women whose sex chromosomes are normal in structure and number (Yunis , 1974) .

Wyanot and Hecht (1971) and Mukherjee, et al. , (1972) recorded the frequency of fluorescent X - chromatin in different tissues . In diploid fibroblasts derived from normal 46 XX female foetus , 45 - 86 % of the nuclei had a single fluorescent X - chromatin body . On the other hand , female amniotic fluid cell cultures from 46 - XX foetuses showed a range of 34 - 76% positive mono X - fluorescent nuclei . Also , in diploid skin fibroblast cultures from three years old girl and those from 30 years old women (both 46 XX) , single fluorescent X - chromatin bodies were encountered in 67 - 71 % of the cells examined . Moreover , fluorescent X - chromatin was present in 63 % of the cells of fibroblast - like cultures derived from normal endometrium and a weakly - fluorescent X - chromatin body was observed in 0.4 - 13 % of buccal mucosal cells obtained from normal women . The authors attributed this wide range of frequencies due to the variation in cell density .

Many investigators have reported that the

the frequency of sex chromatin in buccal mucosa cells is affected by the age of the female. Smith , Marden , McDoland and Speckhard (1962);Taylor (1963) and Frasier , Grud and Farrcll (1964) found that the percentage of chromatin positive cells tended to be low in the first two days of neonatal life then reached the normal levels by the third day . However , Peterson , Jacobson , Teigord , and Fontaine (1966) could not find a significant decrease of sex chromatin frequency in the first four days of life in the new - born females . On the other hand , Glob , Israsena and Hacker (1969) found a gradual increase in X - chromatin frequency during the first few days after birth in association with a decrease of oestrogenic activity . In addition Roy , Das ,Khare , and Roy (1976) found a parallel gradual increase in X - chromatin incidence in female newborns and their mothers during the early few days after delivery . The peaks obtained in their studies were reached on the fifth and sixth days after delivery . These authors suggested that the cause

of this finding was most probably due to the effect of the high levels of steroid sex hormones especially oestrogen , since during this transient period , the newborns have high transplacental steroid hormone carry - over from the mother , and the washing off of this high steroid level from the body of the babies allows X - chromatin incidence to shoot up to peak on the fifth or sixth day .

In contrast, Wegmann and Smith(1964) had attributed the low frequency of X - chromatin positive cells in newborn females in the first day as compared to the subsequent three days , to environmental alternation from amniotic fluid to atmosphere exposure.

Townsend , Case and Lucas (1971) followed the relation between sex chromatin counts throughout pregnancy and the well - known changing levels of oestrogen , progesterone and gonadotrophins . They found a highly significant decrease in sex - chromatin counts of pregnant women as compared to those of women in menstrual cycle , especially in the late period of pregnancy when steroid

levels were high . From this study , the authors suggested that oestrogen had a major role in the activation of X - chromosome during the menstrual cycle and during pregnancy and in both instances , the decrease in sex - chromatin count was correlated more consistently with increased levels of oestrogen than with changes in the level of progesterone and gonadotrophins ; the latter two hormones fluctuate widely in pregnancy and menstrual cycle .

Many years later , Purandare and Chakravirty (1980) reported a lower incidence of X -chromatin frequency in prepubertal , pregnant and menopausal groups in comparison to the mean counts of normal menstruating subjects . As well , they found that the significant low X - chromatim percentage during pregnancy always occurs in the third trimester of gestation , a trimester known to be dominant of sex steroid levels especially that of progesterone . Also , in menopausal women , the sex chromatin frequency was lower than in normal and no fluctuation of X - chromatin frequency was recorded . This could be

attributed to a low level of sex hormones still remaining in the circulation and showing no more cyclic variation .

The frequency of sex - chromatin also fluctuates during regular menstrual cycle (Caratazalli , 1963 ; Blanco and Ramirr , 1965 ; Dokumov and Spasov ,1968 ; Christakos , Simpison and Bohrani , 1969 ; Cavalli , Waldrigue , Stuber and Marcallo , 1970 ; Haggy and Brodrick , 1972 and Purandare and Chakravirty , 1980) These authors reported that the frequency of X - chromatin showed a rhythmic variation during the menstrual cycle with the lowest count in the middle of the period near the ovulation stage , and the highest level at two to three days prior to the next menstrual flow . They attributed this variation to the effect of oestrogen fluctuation as it coincided with the known curve of oestrogen. In contrast , Briggs (1958) ; Davidson (1961) and Brainerd , Meccer and Miles (1965) found no significant difference along the whole phase of the cycle .

Dokumov and Spasov (1967) ,and Becker , Martin and Bourkhus (1973) reported that the percentage of sex chromatin was affected by the administration of exogenous steroid sex hormones .

Caries,Asper,and Smith (1971); Schnear and Naghi (1971) and Mendonca , Zogna , Wejchenberg and Toledo(1984) recorded a low sex chromatin count in a case of congenital virilizing adrenal hyperplasia and reported that the values were resorted to normal ranges by glucocorticoid replacement therapy or with correction of electrolytes and water balance .

Baikie , Garson , Wester and Ferguson (1966) and Wester , Garson , Barnett and Baikie (1967) found that female children with burn had low frequency of sex chromatin in buccal smears . The authors attributed this phenomenon to the possibility of massive amount of glucocorticoids produced in response to the stress reaction of burn . Similarly , a decreased incidence of sex chromatin positive cells in females treated with prednisone

had been reported (Shetty , Sharma and Wahal ,1966)

There are certain biological factors which are known to influence the incidence of sex chromatin . Therkelsen and Petersen (1962) proved that in rapidly dividing tissues , the incidence of Barr bodies was lower than in tissues having rare mitotic activity in which the cells were , on an average , older . Moreover , Mittwoch(1967) and Klinger ; Davis ; Goldhuber and Ditta (1968) reported that the incidence of Barr bodies increased as the cells became more crowded and their nuclei smaller.

Sex chromatin studies have a great value in the diagnosis of sex chromosome anomalies . For example , Sutton (1980) had concluded that a simple cell scraping may aid in the diagnosis of suspected cases of Turner's or Klinefelter's syndrome before karyotyping is made . Obviously , the absence of Barr bodies confirm the diagnosis of Turner's syndrome in females and their presence will diagnose a Klinefelter male .

In addition , X - chromosome study is of great importance for the detection of case of hermaphroditism or pseudohermaphroditism where the anatomical features seem intermediate ; e.g in case of male pseudohermaphrodite , the sex chromatin is negative , while in female pseudo - hermaphrodite there are chromatin positive cells .

Not only the presence or absence of sex chromatin might correlate with X - chromosomal disorders but also its size was affected . For example , in cases of isochromosome , the Barr bodies found in the buccal smears might look larger or smaller than usual according to which isochromosome was inactivated (Ferguson , 1965). The later author also noticed that the size of Barr bodies became smaller than usual in short arm delations of inactive X - chromosome .

In addition , Sohavol and Gaines (1955), tried to use the X - chromatin frequency as a diagnostic means in some diseases . They reported an absence of a characteristic sex chromatin pattern in 19 out of 27 teratomas from females .