BARR BODY STUDIES AND CLINICAL APPLICATIONS

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ABSTRACT

One hundred (100) subjects from among staff and students of Nnamdi Azikiwe University, Nnewi Campus and Nnamdi Azikiwe University Teaching Hospital, Nnewi, were screened for the presence of Barr bodies in buccal smear. 41 males and 59 females (1 a known Turner Syndrome Patient) were studied. Of these, 20 (7 males and 12 females with children, and 1 previously diagnosed Turner Syndrome) subjects, served as positive and negative controls. The buccal smears were stained with Haematoxylin and Eosin (H&E) stain. Results showed that 57 of the 58 female subjects had Barr bodies. No male subject was found to have Barr bodies. None of the positive subjects had more than one Barr body in a cell. A mean Barr body of 22±5.6% standard deviation was obtained for the female subjects. There were slight variations in the Barr body count. Barr body count of married and single female subjects did not show any significant variation at P<0.05. The only female subject without a Barr body had infertility problems though she had no external features of abnormality.

INTRODUCTION

Human somatic cells contain 46 chromosomes, 22 homologous pairs of autosomes and 2 sex chromosomes, XX in the female and XY in the male[1]. These are seen in the normal condition, but there are conditions where the somatic cells are less or more than 46. Such conditions are known as cytogenetic disorders (chromosome mutations). The aberrations underlying cytogenetic disorders may take the form of an abnormal number of chromosomes or alteration in the structure of one or more chromosomes. The normal chromosome count is expressed as 46XX for the female and 46XY for the male. An exact multiple of the haploid is called euploid. If an error occurs in meiosis and a cell acquires a chromosome complement that is not an exact multiple of 23, it is referred to as aneuploidy. The usual causes of aneuploidy are non-disjunction and anaphase lag [1-3].

Cytogenetic disorders can be divided into two: Those involving autosomes and those involving sex chromosomes.

The genetic diseases associated with Karyotypic changes involving the sex chromosomes are far more common than those related to autosomal aberration. Imbalances (excess or less) of sex chromosomes are better tolerated than similar imbalances of autosomes. Autosomal monosomies are usually lethal and rarely occur[3]. Sex chromosome disorders can induce subtle, chronic problems relating to sexual development and fertility, which are often difficult to diagnose at birth. Many are first recognized at the time of puberty. Some are never detected as they do not affect the development of secondary sexual characteristics and fertility in a noticeable manner[4]. It is generally known that only one of the chromosomes is genetically active. The other X chromosome, either of maternal or paternal origin undergoes heteropyknosis and is rendered inactive[5-7]. The inactive X can be seen in the interphase nucleus as a darkly small in contact with nuclear membrane

known as the Barr body or sex chromatin[1,3,5]. Barr bodies are present in all somatic cells of normal females, but they are most readily demonstrated in smear of buccal squamous epithelial cells[8-10]. In the male, Barr body is absent.

The use of buccal mucosa is non-invasive, and easy to obtain, and when combined with molecular techniques is reliable and accurate[9-10]. The use of Barr bodies which can be seen in buccal smear is useful not only in cases of ambiguous genitalia but also in so many other chromosome disorders[11]. Barr body detection or non-detection may be of more diagnostic use than is currently made use of routinely, as a recent study has shown in breast and ovarian cancers[12].

The human abnormalities called Klinefelter's Syndrome and Turner's syndrome both result from the unnatural presence or absence of a Barr body. In the former, a male possesses a Barr body that it should not have while in the latter a female has no Barr body. In Klinefelters Syndrome, which is seen as the most frequent form of male hypogonadism[13], there are two or more X chromosomes and one or more Y chromosomes. 80% are XXY. In Klinefelters Syndrome, Barr bodies are seen because of the extra X chromosome. Turner Syndrome (45 XO) is the most common sex chromosome abnormality in females characterized by hypogonadism[13-14], in addition to other features. It is the second most important cause of primary amenorrhoea after Mullerian duct anomalies[14]. These females are sex chromatin negative, half of them exhibit 45 XO, the other half mosiacism and varied abnormalities of the X chromosome[5]. In multiple X females, more than one Barr body may be seen in each cell. There is increasing tendency of mental retardation in proportion to number of X chromosomes[15]. Presence of Barr body was introduced by the International Olympic Committee in 1968 as a gender verification test but had to be replaced by polymerase chain reception test at the 1992 Barcelona Olympics which is more sensitive.

SUBJECTS AND METHODS

Buccal scrapings were collected from 100 apparently healthy subjects aged between 19 and 52. The subjects were selected from among the staff and students of Nnamdi Azikiwe University/Teaching Hospital, Nnewi, Anambra State, after obtaining their informed consent. Twenty of the subjects were used as controls as follows: seven males and twelve females who are married and have children, served as negative and positive controls respectively, and one known Turner Syndrome case also served as a negative control.

SAMPLE COLLECTION

Samples were collected from the buccal epithelial lining. The subjects were given water to rinse their mouths before collection to prevent contamination with bacteria and food particles. To further restrict bacteria contamination, a light scraping was first discarded and a second deeper scraping taken. The inside of the mouth (cheek) was scraped firmly with the rounded blunt end of a spatula. A new spatula was used for each subject. Subjects who had mouth ulcers or sores in the mouth were excluded. The slightly turbid fluid obtained was immediately smeared upon clean grease-free slides. These were then placed, while still wet, in 95% ethyl alcohol.

Staining was done with Haematoxylin and Eosin.

Sex chromatic lying intra-perinuclearly against the nuclear envelop stained violet. The Barr bodies were counted and expressed as percentage. Only cells which were large with no folding of the nuclear membranes, cells whose nuclear membranes and whose nuclei were not obstructed were counted. The ones with folded or obstructed nuclear membranes or ones in which the nuclear membranes were not intact were excluded.

RESULTS

The age distribution of the subjects is shown in Figure 1. Peak age range was 19 - 24 years (34%). A total number of 41 males and 59 females were studied. Of the forty-one (41) males studied, none was found to possess a Barr body. Of the 59 females studied, one was found to have the Turner Syndrome case. Of the remaining 58, 57 were found to have Barr bodies (98.28%) while 1 (1.72%) had no Barr bodies. A mean of 22% of the total nuclei in positive women possessed Barr bodies. The Barr body count in married and single women did not show a significant difference $P<0.55$. Figure 2 shows the Barr body count in different age groups; 35 - 36 age group showed the highest count of 27%.

DISCUSSION

One hundred subjects were assessed for Barr bodies in buccal smears. In the subject with known Turner syndrome, no Barr body was found in the smear. In this study, one female subject was found to lack the Barr body. Incidentally, she had been married for four years without an issue. Cytogenetic disorders may remain undetected and only detected when associated with infertility[4]. This may well be true with this case work in which the female subject lacking Barr bodies has no outward sign of an abnormality. The secondary sexual characteristics are well developed. The subject is of normal stature and menstruates regularly. The use of Barr bodies from buccal mucosa when combined with molecular technique is reliable and accurate in detecting or excluding cytogenetic disorders. Thus, the subject will require further tests, like karyotyping to confirm or exclude abnormality.

Barr body analysis revealed a specificity of 95% and a sensitivity of 82% for the diagnosis of Klinefelters syndrome, hence provides a quick and reliable screening test, which, however, must be confirmed by karyotyping[12].The subject's husband had been investigated and found to have normal values in semen analysis.

No male subject was found to possess Barr body and this agrees with numerous works which have proved that normal males do not possess Barr bodies. So, one can surmise from this study that only normal males were studied. No female subject had more than one Barr body. The Barr body count of the subjects ranged from 9% to 40% with a mean of 22%. This agrees with a study on Jordanian women[16], which showed that the number of X-chromatin was the highest (approximately 22%) in the <9 - 19 years age group and was the lowest (approximately 10%) in 50 and above years age group. Hagy et al. [17] who worked on variation of sex chromatin in human oral mucosa have their result as between 14% to 56% of squamous cells and an average of 26%. A similar work by Douglass and Beaver[18], gave the range as between 2% and 21%. This wok seems to agree more with the work by Hagy and his co-workers.

The study by Ndubuka et al[19] on the different staining techniques for the demonstration of Barr bodies from buccal smears reported different results using different staining methods. Using cresyl fast violet, an average Barr body count of 25% was reported. For the acetorcein method, an average of 33% was recorded while with Actoorcein 1 Acetocresyl fast violet method, an average of 40% was noticed. An average of 22% Barr body count was observed from this present work using Haematoxylin and Eosin. Choice of method depends on many factors but mostly the sensitivity and availability of such methods. The straining method used is the Harris' Haematoxylin with Eosin as counterstain. H&E is the most commonly used technique in animal histology and routine pathology as the standard histological method [20].

Buccal scrapping as a choice specimen for the Barr bodies has the advantage of being non-invasive and easy to obtain. Other specimens such as skin biopsy and blood films can also be used. Barr body count is a critical exercise in exfoliative cytology. Indeed, it is critical in the sense that upon its result, depends the verdict of male and female patients with doubtful sex

and sexual pathology[7] especially in our environment with limited confirmatory chromosome studies. Indeed, the incidence of cytogenetic disorder may be more prevalent in the general population than is presently realised.

Interest was recently reawakened on the presence of heterochromatic X chromosome in certain breast and ovarian cancers. It is now known that heterochromatic instability is a common but largely unexplored mechanism leading to widespread genomic mis-regulation and the evolution of some cancers[11]. Also, the Y-chromosome is known to play peculiar roles in genetics, sex determination, evolutionary history and, therefore, of significant use in medical, forensic and human evolution. The SRY sex determining region has been linked to gonadoblastoma in intersex patients, testicular germ cell tumours, prostate cancer and other somatic cancers[21]. With the recent happenings in the sports world where some females with extraordinary performances have been found to have male characteristics, Barr body studies (plus PCR) have become even more useful as a screening tool.

RECOMMENDATIONS

We recommend that Barr body studies be made part of investigations for infertile couples when no other abnormality is obvious, as more subjects may be found to lack or possess Barr bodies in the larger population. The studies should also be extended to cancer patients and members of their families for screening, documentation and counselling purposes, as well as to sports women.

CONCLUSION

The incidence of cytogenetic disorders is more prevalent in the general population than is presently known. Barr body studies by cost-saving buccal smears are an important screening tool in our environment where the more sophisticated and expensive polymerase chain reaction (PCR) tests may not be easily accessible.

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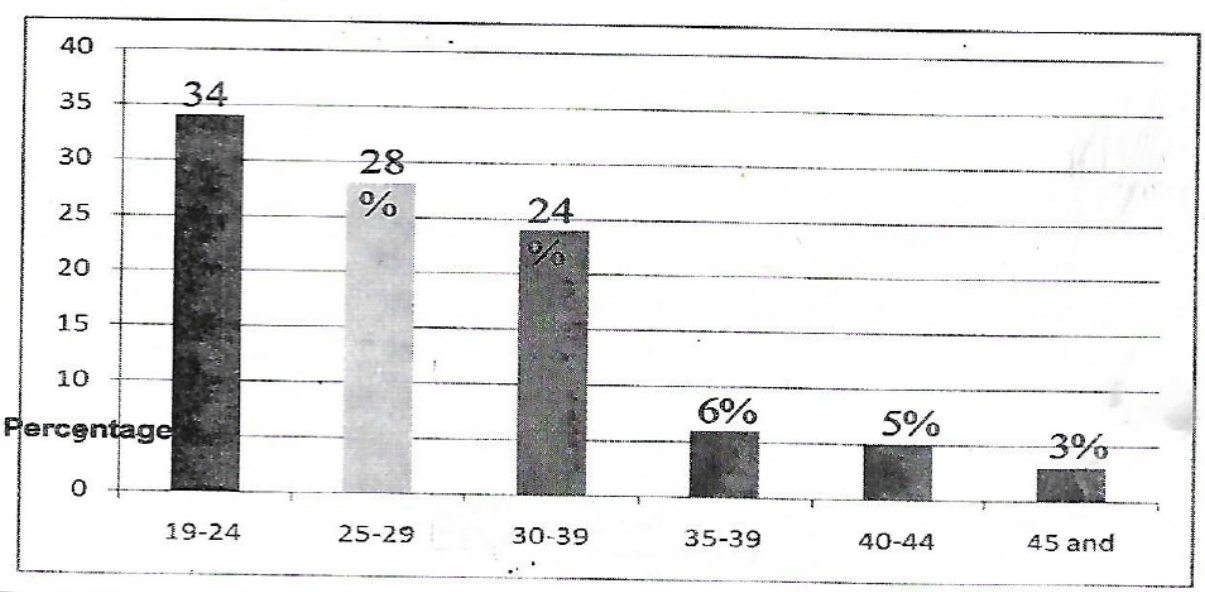

Fig.1: The age distribution of subjects studied
Age range 19-52 years. 19-24 is the highest number (34%).

Average Barr body count (22%)

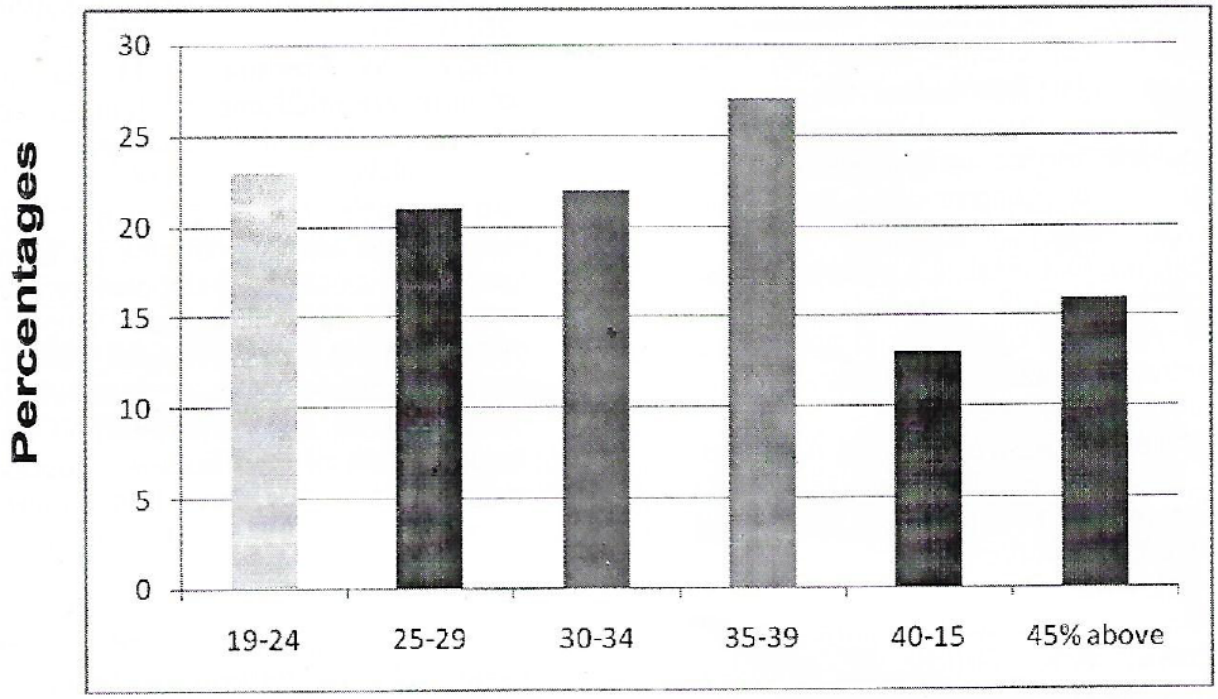

Fig. 2: Barr body count in different age groups
Age groups 35 - 39 showed the highest of 27%.