INTRODUCTION

As civilization developed, man stepped from primitive to advanced life. A systematic way of guidelines was put forth for human beings to lead a secured social life so that people will not be harmed by one’s lifestyle. Any deviated behavior from the normal will be punished or may be kept behind bars depending upon the crime committed; sometimes, the crime conducted may also go unnoticed too. Studies on such abnormal behavior of human beings are carried out to know the reason for the criminal mind but until date many have ended up with very few definitive results. In this context, the association of behavior of a person and sex chromosomal anomalies has become an exciting challenge for the researchers. Sex chromosomal abnormality and its association with psychopath are not only of academic interest but also aim at providing justice to the individual who have been determined as convicts.[1,2]

Before the 19th century, discussion of crime was conducted entirely on moral and philosophical terms. It was only in 1876, an Italian anthropologist Cesar Lombroso published his theories

ABSTRACT

Background: Cytogenetic studies from past decades have shown that interphase cells of female cats contain a densely stained chromatin mass in their nuclei called as Barr bodies (BBs) named after the scientist Murray Barr. BBs are unique chromatin structures formed due to the condensation of the X-chromosome. Many psychopathic disorders originate from defective genes including the multiple X syndromes. Males with extra X-chromosome generally present with severe personality disorder. The present study was conducted to determine the presence of extra X-chromosome in male jail inmates through the detection of BB in peripheral blood and buccal smear.

Materials and Methods: Study included 100 male subjects (fifty jail inmates and fifty controls), after obtaining the consent, peripheral blood smears (PBS) and buccal smears (BS) were prepared and stained using Leishman’s and cresyl violet stain respectively. One hundred neutrophils in PBS and epithelial cells in BS were screened for detection of the BB; accumulated data were tabulated and statistically analyzed using t-test and Chi-square test.

Results: 60% of cases in PBS and 36% in BS showed positivity for the presence of BB in jail inmates as compared to 14% of cases in PBS and none in BS were positive for BB in controls.

Conclusion: Presence of BB in male suggests increased likelihood of criminal tendencies. Further studies are to be carried out to compare the results with karyotyping.

Key words: Barr bodies, criminality, Klinefelters syndrome, peripheral smear and cresyl violet
of criminal behavior with scientific approach to understand the criminal behavior of a person. He also stated physiological theories of criminality focusing on person’s physical form as a mark of criminality. In 1949, Sheldon advanced Lombroso theory and suggested that some crime might be attributed to a chromosomal abnormality. Eventually evidence-based studies established that XYY men are more in offender population when compared with general population. The XYY syndrome was previously considered the “supermale” syndrome where men with this condition were thought to be overly aggressive and more likely to become criminals.[2]

Similarly, in one of the study it was reported that approximately 1% of all individuals institutionalized with mental retardation had an XXY karyotype and approximately half of all mental retardation seen in males originates from a defective gene on the X-chromosome. While the fragile-X site has been implicated in most X-linked mental retardation disorders, other regions of the X-chromosome have also been linked to some mental retardation syndromes. Thus, genes involved in human cognition reside on the X-chromosome.[3] As the syndrome is associated with extra X-chromosome, these patients will show positive sex chromatin material in epithelial cells similar to normal females which are termed as Barr bodies (BB).[4]

The presence of an extra X-chromosome in males is suggestive of Klinefelter syndrome (KS), which affects 167/100,000 men.[5] Individuals with KS generally presents with severe personality disorders, neuroses and psychoses which are usually in the form of paranoid states and schizophrenic reactions. A study was conducted on KS males as a genetic model for psychotic disorders on KS males and compared with an equal number of normal males followed by an examination of brain MRI of these individuals. Ten out of eleven KS males had psychiatric disturbance, four of whom had auditory hallucinations compared with normal, where none of the normal individuals showed any abnormality.[3] Bartholomew studied psychopath, sex chromosome abnormalities and criminal law and stated that multiple X syndromes in men especially in KS showed inadequate behavior when compared with large control hospital group and also showed aggressiveness in behavior which may include criminality.[6]

To examine the crime characteristics of men, the present study was undertaken focusing on the criminal behavior and chromosomal abnormality. We focused on determination of extra X-chromosome in male jail inmates (XXY syndrome) and normal healthy male individuals. Thus, the present study was concentrated on the determination of extra X-chromosome in jail inmates using epithelial cells from buccal smears (BS) and neutrophils in peripheral blood smears (PBS), to detect BB and correlate the same with criminal behavior among jail inmates.

**MATERIALS AND METHODS**

This study was performed on male jail inmates and comparison was carried out with equal number of the normal healthy males or controls without any criminal background.

**Study population**

A total of 100 male subjects (fifty jail inmates from central jail and fifty controls) of above 18 years of age were considered for the study. Clearance was obtained from the Ethical Committee of the Institute to conduct a study on jail inmates as well from controls, and written consent was taken from the higher authorities of central jail. A detailed written consent explaining the need for the study was obtained from the jail inmates and control group. To avoid error, the crime history of the jail inmates was obtained from the jail records. Of 50 inmates, 46 had committed murder, two were convicted for sexual abuse and remaining two had been convicted of robbery.

The identification of the extra X-chromosome was carried out in the study by detecting the presence of BBs in PBS and BS. PBS was prepared by obtaining blood by pricking the finger (capillary blood) with all aspetic precautions. BS was obtained by gentle scraping of the right/left buccal mucosa. BBs were prepared and fixed in 95% alcohol and air dried. PBS and BS were stained using Leishman’s stain and cresyl violet stain, respectively. A total of 100 cells were counted in each smear at 100× magnification for the detection of the BBs. In PBS, neutrophils were analyzed for BB as they are present on the nuclear lobes of the neutrophils as drumstick shaped structure [Figure 1]. In the case of BSs, BBs were found to be attached to the nuclear membrane of epithelial cells [Figure 2]. Statistical analysis was performed using t-test and mean number of BB was calculated using Chi-square test and results were tabulated.

**RESULTS**

Smears prepared from peripheral blood, revealed positivity in thirty jail inmates for the presence of BB out of total fifty cases which contributed to an overall accuracy of 60%. Whereas, in remaining 20 (40%) jail inmates PBS were negative for the same. Similarly, detection of BBs was carried out in BSs stained with cresyl violet stain in fifty jail inmates. Which, revealed positivity for BBs in 18 jail inmates (36%) and depicting negativity in 32 (64%). The average number of BBs present per high power field in case of PBS of jail inmates were around 2–6 cells while; it was reduced to 1–3 cells per high power field while studying BSs [Table 1].

Similarly, the analysis performed on control group showed a decreased number of positivity for the presence of BBs in
Presence of Barr bodies in men indicates criminality? Kulkarni, et al.

PBS contributing around 7 controls (14%), whereas none of the BSs depicted the presence of BBs in controls. The average number of BBs in PBS of controls was 0.32 [Table 2]. The comparison of the average number of BBs in the case of jail inmates and in controls showed insignificant results.

A comparison was performed between jail inmates and controls for detection of BBs using PBS and BS. In the case of PBS, an overall obtained positivity was 60% (thirty jail inmates) while for controls it was reduced to only 14% (7 controls) with an overall positivity of 37% [Table 3]. The comparison between PBS and BS was statistically significant (P < 0.001).

Similarly, the comparison between jail inmates and controls for detecting BBs using BS yielded 36% positivity in jail inmates (18 subjects), and none were positive in case of controls. The comparison between jail inmates and controls for detection of BB using BS showed statistically significant results [Table 4].

DISCUSSION

KS is a genetic disorder which results due to the presence of extra X-chromosome in men. Many times KS goes undiagnosed or is diagnosed late due to its varied clinical presentation. Studies have revealed a correlation between KS and criminal behavior and have determined the presence of 47 chromosomes, XXY disorder by karyotyping which is an expensive method and cannot be performed as a routine screening procedure on a large population.[3-6]

XXY men are found in about 0.8–1% of males hospitalized with schizophrenia, a four-to-five-fold excess over general live birth rates. This may indicate that genes that are overexpressed in the brains of XXY males may also be relevant to schizophrenia and other psychiatric disorders. Since the search for genes leading to susceptibility to major psychiatric disorders has produced inconsistent results. The

![Figure 1: Photomicrograph showing Barr bodies attached to nuclear lobe of neutrophil in peripheral blood smears (Leishman’s stain, ×1000)](image1)

![Figure 2: The presence of the Barr bodies attached to the nuclear membrane of the epithelial cells of buccal smears (Cresyl violet stain, ×1000)](image2)

Table 1: Positivity for Barr bodies in peripheral blood smears and buccal smears of jail inmates

| Smear  | Positive Percentage | Negative Percentage | Number of BB at 100 high power field |
|--------|---------------------|---------------------|-------------------------------------|
| PBS    | 30                  | 60                  | 20                                  |
| BS     | 18                  | 36                  | 40                                  |

PBS: Peripheral blood smears, BS: Buccal smears, BB: Barr bodies

Table 2: Positivity for Barr bodies in peripheral blood smears and buccal smears of control group

| Smear  | Positive Percentage | Negative Percentage | Number of BB at 100 high power field |
|--------|---------------------|---------------------|-------------------------------------|
| PBS    | 07                  | 14                  | 43                                  |
| BS     | 00                  | 00                  | 00                                  |

PBS: Peripheral blood smears, BS: Buccal smears, BB: Barr bodies

Table 3: Comparison for positivity of Barr bodies in jail inmates and controls (peripheral blood smears)

| Groups            | Positive (%) | Negative (%) | Total | χ² | df | P       |
|-------------------|--------------|--------------|-------|----|----|---------|
| Jail inmates      | 30 (60)      | 20 (40)      | 50    | 22.694 | 1 | <0.001  |
| Controls          | 07 (14)      | 43 (86)      | 50    |     |    | (significant) |
| Total             | 37 (37)      | 63 (63)      | 100   |     |    |         |

Table 4: Comparison for the positivity of Barr bodies in jail inmates and controls (buccal smears)

| Groups            | Positive (%) | Negative (%) | Total | χ² | df | P       |
|-------------------|--------------|--------------|-------|----|----|---------|
| Jail inmates      | 18 (36)      | 32 (64)      | 50    | 47.059 | 1 | <0.001  |
| Controls          | 00 (0)       | 50 (100)     | 50    |     |    | (significant) |
| Total             | 18 (18)      | 82 (82)      | 100   |     |    |         |

XXY karyotype may serve as a naturally occurring genetic model to provide clues to the inherited abnormalities that
cause these disorders in individuals with normal chromosomal numbers.\textsuperscript{[5,7]}

It is now known that the XXY karyotype occurs in 1 in 500 live male births and is the most common type of human chromosome anomaly. Unlike chromosomal duplications or translocations on autosomes, the XXY karyotype leads to relatively mild clinically noticeable deviations. This mildness is probably due to inactivation of most genes on the extra X-chromosome. However, there is a class of X-chromosome genes that have homologies on the Y chromosome and tend to escape the normal extra X-chromosome inactivation process, as do some other X-specific genes. It is thought that the characteristic features of KS originate from genes that escape inactivation, and are expressed in excess. Thus, the tall stature, testicular and sex hormone deficiencies, reduction of secondary sex characteristics, and in some cases, breast development in postpubertal Klinefelter’s men may be due to expression of X–Y homologous genes. Similarly, behavioral and cognitive symptoms of KS are likely to result from this class of genes as well. It has been suggested that at least one gene or genes in X–Y homologous regions of the sex chromosomes that escape normal X-inactivation are crucial for language functioning.\textsuperscript{[3,7,8]}

Thus, the aim of this study was to detect the presence of BBs in jail inmates and to co-relate with their criminal behavior. This is the first study to correlate the criminality of jail inmates by detecting their BBs using PBS and BS among the Indian population.

The study has obtained 60\% positivity for BB in jail inmates as compared to normal controls which showed only 14\% in PBS. The comparison between the groups was statistically significant. Whereas the presence of BB in BS was less compared to PBS, but when it was compared within the group, it was statistically significant. A similar study using karyotyping was conducted by Stochholm \textit{et al.} and showed the presence of 47, XXY in a register-based cohort study found moderately increased crime rate in men with KS, whereas few other studies found no such correlation between 47, XXY and increase in crime in affected men.\textsuperscript{[5,7,6]} These findings are in contrast to the observation what we have made. We found good correlation between the presences of BB in jail inmates which further confirms their psychological behavior to commit crime.

BB can also be seen as hyper pyknotic, basophilic, intranuclear structure adjacent to the nuclear membrane in the resting stage of a cell in karyotypic female. It can also be seen adjacent to the nucleolus or be free in the nucleoplasm. It resembles the letter V, W, S or X under electron microscope and measures around 0.8 \(\mu m \times 1.1 \mu m\) in its greatest diameters. BB can be seen in any female cell with large, open nuclei. It is commonly seen in buccal smear, pulp tissue, hair follicle and vaginal smears commonly used for determination of sex. In a smear only 30\% of the cells show BBs. Any nuclear stain can be used for the demonstration of the BBs in BS; most commonly used ones are hematoxylin and eosin (H and E), papanicolaou stain, Feulgen stains, guard stains, cresyl violet, carbol fuschin and fluorescent stains. In H and E and papanicolaou stains, the bacteria stains heavily, hence the BBs are not noticed prominently. Orcein also stains bacteria. Bacterial artifacts can be minimized with acid hydrolysis and thionine staining. Feulgen and guard stains are ideal but need to be standardized every time. Fluorescent stains being more confirmatory for the detection of the BBs but is expensive.\textsuperscript{[4]} In this study, cresyl violet was preferred as it stains BBs more prominently with fewer artifacts and is cost-effective.\textsuperscript{[4]}

The comparison between two methods used for detection of BB (PBS and BS) was carried out. Number of cases for the presence of BB was high in PBS method when compared with BS. It is suggested that PBS is the most accurate and reliable one for detection of BB. Comparison between number of BB present in the case of jail inmates and normal healthy male groups revealed that there was no much difference between the two groups for the average number of BB but the number of positive cases for BB was less in controls compared to jail inmates. This suggests that probably the number of BB does not play an important role in the determination of extra X-chromosomes. Even though presence of extra X-chromosome in men can be diagnosed using various methods but detection of BB in PBS is simpler, reliable, easy and less expensive procedure.\textsuperscript{[10]} Presence of 60\% of the BB in PBS in jail inmates has revealed definite association between the presences of extra X-chromosome in men with criminality. It is well accepted that X-chromosome bears around 1100 genes which are responsible for the normal functioning of brain and testes. The presence of inactive extra “X” chromosome may contribute for the deviated behavior among the KS persons.\textsuperscript{[11-13]} With the adequate numbers of studies in large group of population one can find out the reason for the criminality in KS. The detection of BB in males and its correlation to criminality has proved that there is an association between genetic alteration and antisocial behavior of a person. This alteration has to be taken into consideration during trial of a convict.

**CONCLUSION**

In this study, the jail inmates showed increased number of positivity for BB, which is suggestive of extra X-chromosome. This preliminary study revealed presence of BB in jail inmates which can be correlated with their criminal behavior but further confirmation needs to be done for the presence of the extra X-chromosome by performing karyotyping. Nevertheless, detection of BB using PBS and BS appears to be a simple, cost-effective and appropriate screening method to detect XXY syndrome in criminals. Presence of BB in jail inmates will further confirm their criminal mind, which has
been suspected to have committed crime and it also confirms the crime in victims who is already a convict. Authors intend to further study and correlate the presence of BB with karyotyping to confirm our preliminary findings.

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Conflicts of interest
There are no conflicts of interest.

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