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The Effectiveness of Barr Body in the Determination of Females Sex

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Authors' contributions
This research was conducted in collaboration between the two authors. The authors involved in the study design, protocol writing, results interpretation and final manuscript draft, reading and approval. Author RM S, SA M, H A. A, M G. A and D A. A managed the field data collection. Authors Y F. A, M S. A, M K. A and W K. A preformed the laboratory techniques and statistical analysis. All authors read and approved the final manuscript

Abstract: *Medical Genetic is the branch of medicine that involves the diagnosis and management of hereditary disorders. This includes studies of inheritance, mapping disease genes, diagnosis and treatment, and genetic counseling. Demonstration of nuclear sex play initial role in sexing of the individual. Barr body is condensation of chromatin present at the nuclear of cell in female individuals. The objectives is to evaluate the reliability of blood film smear barr bodies for sex determination and to find out the percentage of the positive and negative cases. This was retrospective and prospective study to evaluate the efficiency of barr Body in blood smear for determination of female sex. Including 32 female blood film samples stained by Giemsa stain and diagnosed according to criteria of Barr body identification. Laboratory analysis of the result reveal that the positive were 84.4 %, negative samples were 15.6 %, the sensitivity was 96 % and specificity was 55 %. Regarding our results that the barr body blood film is highly sensitive that can be used as diagnostic method for different purpose such as sex determination in forensic medicine, and in rapid diagnosis of sex for urgent cases, but this method was less specific. Therefore we recommended using of barr body blood film as diagnostic method but can be confirmed by more modified method. Also giemsa stain can be modified to be more specific for barr body determination.*

Keywords: Barr Body Determination; Giemsa Stain; Kingdom Saudi Arabia

1. INTRODUCTION

A microanatomist from London Ontario Murray Barr, n the late 1940s, discovered a mark of sex chromosome status in bodily tissues, what came to be known as the 'Barr body'. This discovery porvided an important diagnostic technology to the clinical science community engaged with the medical interpretation and management of sexual anomalies. It is important to identify the true underlying sex in those whose bodies or lives were sexually anomalous (Fiona Alice Miller, 2006). The barr body was named at the discover of MurrayBarr in 1949.barr body is inactive X chromosome in a female somatic cell, the inactivity due to the process called lyonization, (Lyon, 2003). This process occurs in species in which the sex is determined by the presence of the Y (including humans) or W chromosome rather than the diploidy of the X. The Lyon hypothesis in 1961in cells with multiple X chromosomes, one is inactivated during mammalian embryogenesis, the inactivation occurs early in embryonic development random in mammals, (Brown, 1997). Except in marsupials and in some extra-embryonic tissues of some placental mammals, in which the father's X chromosome is always deactivated, in humans with more than one X chromosome, the number of Barr bodies visible at interphase is always one fewer than the total number of X chromosomes. Barr bodies can be seen on the nucleus of neutrophils (Lee, 2003) (Fig 1.1).

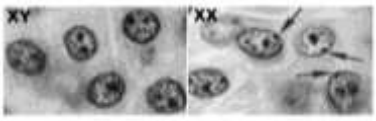

Fig (1.1) Sex determination was based on lack of barr bodies in maria`s cheek cells, indicating XY chromosomes.

Gregor Mendel is known as the Father of Genetics. There was, little awareness of Gregor's work during that time. Also, in this period Haeckel correctly predicted that the heredity material was located in the nucleus. Miescher showed the material in the nucleus was a nucleic acid. Chromosomes as units carrying genetic information was also discovered around this time (AnanyaMandal, 2018).

A chromosome is a deoxyribonucleic acid (DNA) molecule with part or all of the genetic material (genome) of an organism. Most eukaryotic chromosomes include packaging proteins which, aided by chaperone proteins, bind to and condense the DNA molecule to prevent it from becoming an unmanageable tangle (Hammond CM, Groth A, March 2017), (Wilson, John 2002).The basic number of chromosomes in the somatic cells of an individual or a species is called the somatic number and is designated 2n. Each human cell thus contains 46 chromosomes in 23 pairs. The gametes or ovum produced by the female ovaries and the sperm produced by the male testicles, however, contain only 23 chromosomes. (Ananya Mandal 2018). DNA testing of buccal smears replaced nuclear sexing in the 1980s, at that time it was used for, a gender verification test for female athletes, the same discrimination continued until the Olympic Games in Sydney in 2000, when gender verification was finally abandoned (Ferguson-Smith, 2001). Giemsa stain is a classic blood film stain for peripheral blood smears and bone marrow specimens, is also used to visualize the chromosomes (Shapiro, 2007). Labpedia. Net (2020), published the indications for barr body test; that used for delayed puberty, for turner and Klinefelter syndromes, don't take the sample during first week of the life of newborns, or during adrenocorticosteroids or estrogen therapy, 40-60% of the cells show identifiable barr body. Leishman stain is named after its inventor, the Scottish pathologist William Boog Leishman, it is a version of the Romanowsky stain, and is thus similar to and partially replaceable by Giemsa stain, Jenner's stain, and Wright's stain (Biotech Histochem, 2011).

2. DEFINITION OF STUDY AND STUDY AREA

This was a prospective, retrospective study to evaluate the efficiency of Barr Body in blood smears for determination of female sex. The study was conducted during the period from January- April 2019.

Thirty two female student blood smears samples were retrieved from the hematology laboratory of the college of Applied medical Sciences in Hafar Al-Batin city.

Hafer Al-Batin is Saudi Arabian city in the Eastern Province. It is located 430 km north of Riyadh. In 2010, Hafer Al-batin, had more than 35 villages in it is suburban area and the total population reached 389,993 to 600,000.

3. BLOOD COLLECTION AND PREPARATION OF SLIDE AND INDEX FINGERNAIL:

New microscope slides were used, the tip of the middle or ring finger was pricked with lancet , then place a small drop of blood in the very clean slide. Then spreading the material on slide and let it to dry and the smear fixed in methanol for 30 seconds.

4. GIEMSA STAINING TECHNIQUES:

The fixed blood smear was stained with Giemsa stain and allowed to stand for 10 minutes. After staining, the slide was washed with water to remove the excess stain. Finally, the slide was kept for air-drying and then observed under the microscope (100X).

Interpretation:

Barr bodies are densely stained condensed chromatin masses adjacent to the nuclear membrane. They can be plano-convex, biconvex, triangular, spherical, or rectangular in shape when observed under ordinary microscope in oil immersion.

The presence of Barr Body smear positive (+ve) (Fig 4.1).

And the absence of Barr Body smear negative (-ve) (Fig 4.2).

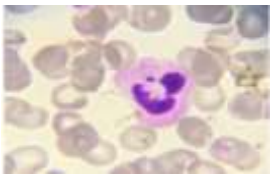

Figure (4.1): positive control Barr body

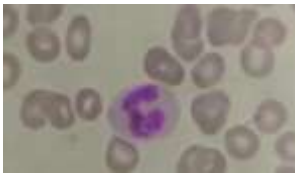

Figure (4.2) negative control Barr body.

5. RESULTS

In this study 32 female samples were included, in which the blood films were made and stained with giemsa stain and the barr body was assessed in each specimen.

5.1 The diagnosis of barr body among the study population:

Out of 32 female cases, most of cases were positive for barr body 27 (84.4%), and the negative cases for barr body were 5 (15.6%) (figure 5.1).

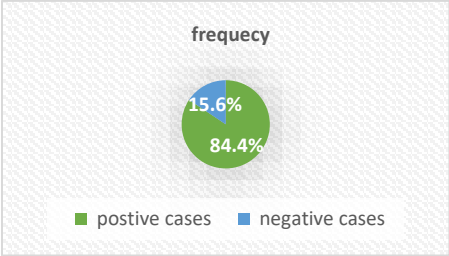

Figure (5.1) The diagnosis of barr body among the study population

5.2 The sensitivity of barr body blood film test:

The sensitivity of barr body blood film test was 96% (table 5.1).

Table 5.1: The sensitivity of barr body blood film test

Test	Sensitivity
Barr body blood film	96%
control	100%

5.3 The specificity of barr body blood film test:

The specificity of barr body blood film test was 55% (table 5.2).

Table 5.2: The specificity of barr body blood film test

test	Specificity
Barr body blood film	55%
control	100%

5.4 The positive predictive and negative predictive values of Barr body test :

The positive predictive value was (87%), and negative predictive values was (83%).

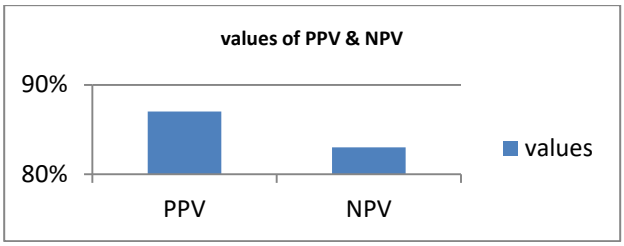

Figure (5.2) The positive predictive and negative predictive values of Barr body test

5.5 Summary of the diagnosis, sensitivity, specificity, PPV and NPV of Barr body blood film test:

	Diagnosis		Sensitivity	Specificity	PPV	NPV
	positive	negative				
Barr body blood film	27	5	96%	55%	87%	83 %
Control	32		100%	100%	100 %	100 %

6. DISSICUSION

Demonstration of nuclear sex play initial role as far sexing of the individual is concerned the sex chromatin or barr body, which is condensation of chromatin present at the nuclear of cell in female individuals.

Our study was showed 27 (84.4%) presence of Barr body and 5 (15.6%) absence of Barr body in female samples of blood film stained by Giemsa stain.

Our positive result agrees with (Anoop UR (2004). They confirmed that various cytologic studies prove the presence of condensed deeply stained chromatin material in nuclei of female cats in 1940's which was later termed as Barr-body by Murray Barr. These cells found to be present only in females which can be used as a vital tool for determination of sex of the individual.

The 15.6% absence of Barr body because the reducing number or presence or absence of Barr body in females may depend on various factors such as menstrual cycle, pregnancy, Turner's syndrome and ovarian dysgenesis which show male sex chromatin pattern. Also the use of nonspecific materials look like Bar body like RNA materials and debris which make it difficult to differentiate the barr body.

Ambika Murugesan, *et al* (2018), they studied the mythological method of Barr body expression in Dental Pulp tissue, they used Giemsa stain and other stains for Barr body. They found that Barr bodies are seen in 40% of female cells. Barr bodies expression can be determined by the use of nuclear stains such as H&E, thionine, Papanicolaou, Feulgen, cresyl-violet, Giemsa, aceto-orcein, and under fluorescence such as acridine orange.

Shyam Prasad Reddy (2012), published determination of individuals have the possibility of carrying the primary sex chromosomes of both the sexes, so this study was mainly done how to differentiate the expression of Barr-body in females and males in order to set criteria for identification. In this study, authors have considered sample size of 20 each of male and female. The samples were fixed in 100% alcohol for 15 min and stained with acridine orange stain and viewed under confocal microscope. Results obtained found to have in males 0%-3% were Barr-body positive and in females 18%-72%. Concluding from this study is that Barr-body found to be positive in both sexes with or without syndromes; it is needed to stain it appropriately and to visualize using proper microscope because Barr-body has the capacity to polarize.

Androgens exert their effects in mediating the development of the normal male phenotype via a single receptor protein, the androgen receptor (AR), which is encoded on the X chromosome. Abnormalities that alter the function of this receptor result in a range of abnormalities of male phenotypic development. These phenotypes range from that of normal females (complete testicular feminization, complete androgen insensitivity) to those that are characterized by only minor degrees of under virilization and/or infertility (Am J Obstet Gynecol, 2000).

Ivan Suazo Galdames (2011). This study was done in how to evaluate the effect of influence of high temperature on pulp tissue and its ability after subjected to high temperature in determination of sex. In vitro study of 50 teeth, 25 each of males and females were taken. Obtained teeth was placed in 10% formalin at a temperature of 34°C and relative humidity of 100%. Then teeth were subjected to varying degree of heat, after this coronal pulp tissue was extracted and processed and analyzed under a trinocular microscope Olympus. Results were positive for females in the tooth subjected to temperature until 400°C, whereas tooth subjected to further high temperature it was not possible to find any viable tissue for analysis. This correlates to the fact that tooth is well-preserved within oral cavity so that only minimum heat dissipates to the

tooth which makes dental pulp tissue to withstand and helps for the diagnosis of sex in forensic medicine.

Roeltgen and Zinn (2007), they studied the loss of the heterochromatic X chromosome (Barr body) in certain breast cancer and ovarian cancer. The Mitotic segregation errors commonly explain the loss of the inactive X chromosome (Xi)), but compromise of Xi heterochromatin in some cancer may signal broader deficits of nuclear heterochromatin. The debated link between BRCA1 and Xi might reflect a general relationship between BRCA1 and heterochromatin, which could connect BRCA1 to both epigenetic and genetic instability. We suggest that hetero chromatic instability is a common but largely unexplained mechanism, leading to widespread genomic misrepresentation and evolution of some cancers.

Nirmal Das (2004), studied Forensic odontology he found it useful in identification of age and sex of patients. Sex of the individual can be determined based on the morphology of canines. Apart from this method, it can also be determined by using X and Y chromosomes in the cells which are inactive. X chromatin in its inactivated form is present as a mass against the nuclear membrane in females is known as Barr body as it was first named by Barr and Bertem (1949). These Barr bodies are present in 40% of females who are considered as chromatin positive and absent in males who are considered as chromatin negative.

Homologous pairs of chromosomes usually replicate in synchrony. However, one of the X chromosomes is always late in replicating. This is the inactive X chromosome that forms the sex chromatin or so-called Barr body, which can be visualized during interphase in female somatic cells. This used to be the basis of a rather unsatisfactory means of sex determination based on analysis of cells obtained by scraping the buccal mucosa—a 'buccal smear'(Peter Turnpenny (2017).

Turner Syndrome (45X), this condition was first described clinically in 1938. The absence of a Barr body, consistent with the presence of only one X chromosome, was noted in 1954 and cytogenetic confirmation was forthcoming in 1959. Although common at conception and in spontaneous abortions, the incidence in liveborn female infants is low, with estimates ranging from 1 : 5000 to 1 : 10,000 (Peter Turnpenny, 2017).

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7. CONCLUSION

Regarding our results that the barr body positive in 84.4% of cases and the sensitivity was 96%, barr body blood film is highly sensitive that can be used as diagnostic method for different purpose such as sex determination in forensic medicine, and in rapid diagnosis of sex for urgent cases.

This method less specific depending on our result that the negative cases for barr body was 15.6% and specificity was 55%.

Therefore we recommend using of barr body blood film as diagnostic method but can be confirmed by more modified method. Also giemsa stain can be modified to be more specific for barr body determination.

ETHICAL APPROVAL

Tissue blocks were used (the samples from the Bank), numbered samples, no patients' name.

Ethical approval for this study was obtained from the research ethical committee from the Gezira State Ministry of Health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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