BIOLOGIA (PAKISTAN)
June, 2018, 64 (1),101 -104

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Sex determination through Barr bodies of neutrophils in Humans and Dogs

MUSHTAQ HUSSAIN LASHARI[1*], RABIA HAMEED[1], UMER FAROOQ[2], MUSADIQ IDRIS[2] & ZIA UR REHMAN[2]

[1] Department of Life Sciences, The Islamia University of Bahawalpur, Pakistan
[2]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan

ABSTRACT

Blood samples of healthy adult humans (n = 40) and dogs (n = 30) grouped as males and females, were collected aseptically through appropriate measures. Thin blood smears were stained using Field stain 'A' and 'B'. The PMNs were counted and observed for Barr bodies attached to them. Only the neutrophils with terminable lobes were examined and counted. The mean value ± SE of the Barr bodies was calculated and the difference between males and females of both species was analyzed through independent T-test. The mean ± SE values for Barr bodies was significantly higher ($P \leq 0.05$) in human females (7.7 ± 0.63) as compared to their male counterparts (2.2 ± 0.38). However, statistically non-significant ($P \geq 0.05$) result was noticed between female and male dogs being 1.8 ± 0.32 and 0.8 ± 0.24, respectively. In a nutshell, it is concluded that both the females of humans and dogs have a higher occurrence of Barr bodies on their PMNs as compared to their male counterparts. However, sex determination in humans through this cyto-diagnostic technique is quite certain and precise as compared to that in dogs.

Key words: Barr bodies, Polymorph nuclear neutrophils, sex determination

INTRODUCTION

Sex chromatin (Barr body) is a small clump of chromatin usually seen at periphery of female nuclei in certain tissues such as that of cornea, oral cavity, vagina and fibroblasts (Brahimi *et al.,* 2013; Tupakula & Thyagaraju, 2014). In stained blood smears, they are seen as minute 'drumsticks' on the Polymorph Nuclear Neutrophils (PMNs). For almost a decade, the presence / absence and frequency of occurrence of these drumsticks (also known as 'nucleolar satellites' or 'sex chromatin' or 'Davidson's Bodies' or 'Barr Bodies) on the PMNs have provided a clinical tool for assessment of gender both in humans and in animals (Miller, 2006; Brahimi *et al.,* 2013; Ajuogu *et al.,* 2014). These drumsticks/Barr bodies of the PMNs were first identified in 1949 by Barr and Bertram while working on effects of stress on nervous system. Later on, Davidson & Smith (1954) related these nuclear appendages to sex chromatin. In their original observation, Davidson and Smith classified five types of nuclear appendages in mature neutrophils: drumsticks, sessile, nodules, small clubs, minor lobes and racket formation. They considered only 'drumsticks' to be related to the sex chromatin. It is now established that the sex chromatin seen as darkly staining mass at the nucleus of all non-dividing cells of genotypically females represents the heterochromatin of the inactivated X-chromosome (Brahimi *et al.,* 2013; Ajuogu *et al.,* 2014).

In human forensic medicine, presence/ absence of Barr bodies and their frequency on various tissues has gained a strong footing as a reliable cyto-diagnostic technique. Previous work has clearly established this technique for sex determination in the dental pulp of human cadavers (Suazo *et al.,* 2010; Priyadharscini, & Sabarinath, 2013). Similarly, the reliability of this cyto-diagnostic approach through Barr bodies on PMNs has been reported for various breeds of goats (Okonkwo *et al.,* 2010), cattle (Ajuogu *et al.,* 2014) sheep (Barjatiya *et al.,* 2016) and various laboratory animals (Cadar, 2009). However, to the best of our knowledge, no such work has yet been reported from Pakistan. The present work, is thus, aimed towards the assessment of the presence/absence and frequency of occurrence of Barr bodies as a confirmatory guide towards sex determination in humans and dogs.

Author's Contribution: M.H.L., Developed research concept; **R.H.** Did Lab work & Data acquisition; **U.F.,** Statistical analysis & manuscript preparation; **M.I.,** Data organization & Interpretation; **Z.U.R., Sample collection & manuscript preparation**
***Corresponding author**: *mushtaqlashary@gmail.com*

MATERIALS AND METHODS

Study Design and Population

The study was approved by the Directorate of Research, Innovation and Commercialization of the Islamia University of Bahawalpur (IUB), Pakistan through its Department of Life Sciences and University College of Veterinary and Animal Sciences (UCV&AS). For human subjects (n = 40), apparently healthy students of the Doctor of Veterinary Medicine and, of Bachelor in Life Sciences (aged 18 – 20 Yrs) were incorporated in the study apropos to a written consent and were divided into male and female groups (n = 20 each). For dog population (n = 30), stray dogs were incorporated in the study and grouped likewise (n = 15 each).

Blood Collection

In humans, blood was collected aseptically from either the cephalic or the median cubital vein of each subject as per the directed guidelines of the World Health Organization (WHO, 2010). The blood was collected in lavender-topped vacutainers (EDTA) for preservation till processed in next 08 hrs. Regarding dogs, the blood was collected from saphenous vein after appropriate restraining by professional personnel. Samples were brought to the Physiology laboratory of UCV&AS, IUB for further analysis.

Slide Preparation and Staining

A drop of whole blood was used for making a thin smear which was air dried, fixed in absolute alcohol and stained using Field's Stain (SDL, Pakistan) (Chunge *et al.,* 1989). Field's stain consists of two parts - Field's stain 'A' is methylene blue and Azure 1 dissolved in phosphate buffer solution; Field's stain 'B' is Eosin Y in buffer solution.

Observations

Dried and stained slides were observed under the microscopic magnification of 100X (oil immersion lens). Total neutrophils were counted throughout the slide and those having Barr bodies attached to them were counted separately. Only neutrophils with identifiable terminal lobes were examined. Terminal lobes correspond to the two end lobes in the linear array of lobes that form the neutrophil nucleus (Karni *et al.,* 2001).

Statistical Analysis

Statistical analysis was conducted through Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA). Descriptive analysis was implied and the difference between male and female humans and dogs was analyzed through independent T-test (Verma & Adinarayan, 2017).

Results and Discussion

The present study is the first of its kind being reported from Pakistan with an aim to assess gender in humans and dogs through presence/absence and frequency of Barr bodies on PMNs. Owing to the paucity of literature on similar work, the comparisons of our results have been made with those of other species. Though the sample population is small, however, it is a preliminary study which provides a baseline data regarding a vital cyto-diagnostic technique.

The result of Barr bodies on the PMNs of human males and females is presented in Table 1. The mean ± SE values for Barr bodies was significantly higher ($P \leq 0.05$) in human females as compared to their male counterparts being 7.7 ± 0.63 and 2.2 ± 0.38, respectively. The percentage of occurrence ranged from 5.5 to 11.1 in all the females, and from 0 to 8.4 in males.

The literature review reveals that the cyto-diagnostic approach of detecting presence/absence of Barr bodies and their frequency on various tissues such as that of cornea, oral cavity, vagina, fibroblasts and stained PMNs is gaining popularity in human forensic medicine (Suazo *et.al.,* 2010; Brahimi *et al.,* 2013; Priyadharscini & Sabarinath, 2013; Tupakula & Thyagaraju, 2014). It has been established that human females have higher Barr bodies than males. However, various workers have reported varying percentages. A lower percentage has been reported as 2.4 to 5.1 and 0.4 to 1 for human females and males, respectively (Barjatiya *et al.,* 2016). Our results are in line with a work which has reported a mean ± SE value of 7.7 ± 0.5 Barr bodies in human female PMNs (Chaterjee, 2014). Similarly, another study has revealed a percentage of 7.6 and 3.9 for true drumsticks in human female and male PMNs (Tupakula & Thyagaraju, 2014) respectively. Yet another study from India has reported mean values of 5.4 ± 2.6 and 2.1 ± 1.8 for human female and male PMNs, respectively (Verma & Adinarayan, 2017) which are in accordance to our study. While studying the dental pulp tissue, it has been reported that mean value of 20.4 ± 0.4 Barr bodies were noticed in

human females, whereas no positive cells with drumsticks were noticed at all for males (Suazo *et al.,* 2010). Though some old literature has revealed a complete absence of such chromatin bodies in male tissues (Briggs, 1958), however, our and most other works have reported presence of these bodies in males as well though their frequency of occurrence is too low.

The result of Barr bodies on the PMNs of male and female dogs is presented in Table 2. A statistically non-significant (P ≥ 0.05) result was noticed between female and male dogs being 1.8 ± 0.32 and 0.8 ± 0.24, respectively. The percentage of occurrence ranged from 0 to 4.5 in all the females, and from 0 to 3.6 in male dogs.

Along with humans, the patterns of canine chromosome have also been studied extensively both in vivo and in vitro (Fraccaro *et al.,* 1965; Cadar, 2009). Previous work has revealed that the results regarding presence/absence and frequency of occurrence of drumsticks in various canine tissues are almost same as those for humans (Fraccaro *et al.,* 1965; Cadar, 2009). In a comparative study, it has been concluded that sex dimorphism on this cyto-diagnostic approach is very certain in dogs as compared to that in white mouse, rat, guinea pig and rabbit (Cadar, 2009). This study reported an average percentage of 5.75 and 0.05 for female and male dogs, respectively which is slightly different from our study being 2.15 and 1.01 for female and male dogs, respectively. In contrast to our study, literature reveals a statistically significant difference between male and female dogs (Fraccaro *et al.,* 1965; Cadar, 2009). A plausible justification could be the less number of population/samples in our study. Results indicate, in a nutshell, that both the females of humans and dogs have a higher occurrence of Barr bodies on their PMNs as compared to their male counterparts. However, sex determination in humans through this cyto-diagnostic technique is quiet certain and precise as compared to that in dogs. Future horizons include better cyto-genetic analyses with a higher population, and in various species. These studies need to be elaborated in correlation to various genetic anomalies as well.

Table 1. Comparison of Barr bodies in human males and females

Groups	Neutrophils Counted	No. of Barr bodies	% of Barr bodies	Mean ± SE	Range	Min. Value	Max. Value	P value
Males (n = 20)	1819	45	2.4	2.2 ± 0.38	0-7	0	7	0.00
Females (n = 20)	1741	154	8.8	7.7 ± 0.63	4-16	4	16	

Significant at P ≤ 0.05

Table 2. Comparison of Barr bodies in male and female dogs

Groups	Neutrophils counted	No. of Barr bodies	% of Barr bodies	Mean ± SE	Range	Min. Value	Max. Value	P value
Males (n = 15)	1187	12	1.01	0.8 ± 0.24	0-3	0	3	0.13
Females (n = 15)	1298	28	2.15	1.8 ± 0.32	0-4	0	4	

REFERENCES

Ajuogu, P. K., Yahaya, M. A. & Ndubuisi, N. P. 2014. The X-chromatin (Barr Bodies) status and deferential white blood cell count of the Nigerian indigenous trade bull cattle breeds. *J Anim. Sci. Adv*. **4**: 812-816.

Barjatiya, R., Geeta, H., Jaskaran, J., Savita, M. & Prakarti, U. A. 2016. Comparative study of drumstick pattern in Polymorph-Nuclear neutrophil leucocytes in common mammals. Indian *J. Res*. **5**: 39-41.

Barr, M. L. & Bertram, E. G. 1949. A morphological distinction between neurons of the male and female, and behavior of the nuclear satellite during accelerated nucleoprotein synthesis. *Nature*. **163**: 676-78.

Brahimi, M., Affaf, A., Hassiba, L., Hadjer, B., Soufi, O. & Mohamed, A.B. 2013. Can sex be determined from a blood smear?. *Turk. J. Hematol*. **30**: 53-57.

Briggs, D.K. 1958. The individuality of nuclear chromatin with particular reference to polymorphonuclear neutrophil leukocytes. *Blood*. **13**: 986-1000.

Cadar, M. E. 2009. Cytological estimation of sexual dimorphism by microscopic examination of peripheral blood in some laboratory animals. Bull. Univ. Agri. Sci. Vet. Med. Cluj-Napoca. *Anim. Sci. Biotech*. **64**: 1-2.

Chaterjee, S. 2014. Reliability of sexual dimorphism in blood. Indian J. Physiol. *Pharmacol*. **58(4)**: 400-402.

Chunge, C. N., Ngige, S., Bwibo, C. R. A., Mulega, P.C., Kilonzo, J. F., Kibati, F., Owate, J. 1989. A rapid staining technique for *Leishmania* parasites in splenic aspirate smears. *Ann. Trop. Med. Parasitol.* **83**: 361-364.

Davidson, W. M & Smith, D. R. 1954. A morphological sex difference in the polymorphonuclear neutrophil leucocyte. *Brit. Med. J*. **2**: 6-11.

Fraccaro, M., Avsson, I., Hultén, M., Lindsten, J., Mannini, A., & Tiepolo, L. 1965. DNA replication patterns of canine chromosomes in vivo and in vitro. *Hereditas*. **52**: 265-270.

Karni, R. J., Wangh, L. J. & Sanchez, A. J. 2001. Nonrandom location and orientation of the inactive X chromosome in human neutrophil nuclei. *Chromosoma*. **110**: 267-274.

Miller, F. A. 2006. Your true and proper gender: the Barr body as a good enough science of sex. Studies in History and Philosophy of Science Part C: Stud. Hist. Philos. Biol. *Biomed. Sci*. **37**: 459-483.

Okonkwo, J. C., Omeje, I.S. & Egu, U. N. 2010. Identification of X-chromatin and determination of its incidence in Nigeria goat breeds. *Livestock Res. Rural. Dev*. **22**: 1-6.

Priyadharscini, R. A. & Sabarinath, T. R. 2013. Barr bodies in sex determination. *J. Forensic Den. Sci*. **5**: 64-69.

Suazo, G. I., Roa, H. I. & Cantín, L. M. 2010. Sex chromatin in dental pulp. Performance of diagnosis test and gold standard generation. *Int. J. Morphol*. **28**:1093-1096.

Tupakula, S. & Thyagaraju, K. 2014. Sexual dimorphism in morphology and morphometry of neutrophil drumsticks. *Int. J. Anat. Res*. **2**: 720-726.

Verma, R. & Adinarayan, M. 2017. Reliability of neutrophilic nuclear appendages in morphological sex differentiation. *Int. J. Med. Sci. Public Health*. **6**: 783-786.

World Health Organization WHO guidelines on drawing blood: best Practices in phlebotomy. 2010.