Short Communication

Sequence Diversity of Interferon Beta-1 Gene in Dromedary Camel Breeds of Pakistan

Tanveer Hussain, Masroor Ellahi Babar*, Akhtar Ali and Fiaz Hussain

Virtual University of Pakistan, Lahore, Pakistan

A B S T R A C T

With the imminent danger of global climate change threatening the Earth's biodiversity, the camel proved to be a unique animal with physiological features. These attributes of the camel instigate us to explore its genetic architecture. Pakistan boasts a camel population of about 20 different breeds and a total of one million head. However, till date, scarce genetic data is available on Pakistani camel's genomics. In efforts to explore the genetics of the camel, we chose to study the Inteferon beta 1 (IFNβ1) gene in in Mareecha camel and non-descript camel breeds in Pakistan. We amplified the IFNβ1 gene through PCR, followed by sequencing. All sequences were aligned and edited through Codon Code aligner and finally a 364 bp portion was selected for further analysis. Dual peaks/heterozygous conditions were identified at positions c.36 (A/C), c.87 and c.225 (Y, T/C). The phylogenetic tree was constructed through MEGA 6.1 software package using the Neighbor Joining method with a bootstrap value of 1000. Finally, the dromedary camel sequences from Pakistan were compared with corresponding genetic sequences of other animal species available on NCBI including European bison, Indian bison, Tibetan antelope, wild yak, domestic goat, sheep, water buffalo and cattle for biological positioning of dromedary camel. The results reconfirmed the classical biological classification.

Pakistan is enriched with a variety of livestock resources, and the camel is one of the most significant livestock assets in the arid and hot regions of the country. The camel is known as the "Ship of the Desert" due to its unique versatility and ability to survive in arid as well as semi-arid areas (Ahmad *et al.*, 2010). Camel (*Camelus dromedaries*) is an animal of enormous economic value. Camel provides useful products and services e.g. milk, meat, wool and drought power to mankind (Saad *et al.*, 2017). Camels have high rate of tolerance ability adjacent to a very severe, harsh and dehydration-prone environment (Al-Soudy *et al.*, 2018). There are two types of camels found in Pakistan, riverine and mountain/hilly. Pakistan has 21 documented camel breeds. Seven breeds are present in Balochistan province of Pakistan i.e. Kachhi, Brahvi, Kharani, Makrani, Lassi, Pishin, Rodbari, whereas, in Khyber Pakhtunkhwa, four breeds i.e. Gaddi, Khader, Ghulmani and Maya, in Punjab, there are five breeds i.e. Bagri, Barela or Thalocha, Cambelpuri, Kala-Chitta and Mareecha and four breeds are documented in Sindh province of Pakistan i.e. Dhatti, Khari, Larri or Sindhi and Sakrai (Ali *et al.*, 2018). Out of these, Mareecha, Dhatti, Larri, Sakrai are riverine type while Kohi, Cambelpuri are

mountain/hilly sort of animals (Nazir *et al.*, 2017). Evidence suggests that when considering the ability of survival of a certain species, genetic diversity at the level of the major histocompatibility complex (MHC) is very important in vertebrates. The MHC consists of a set of cell surface molecules, whose major function is to bind to fragments from pathogens and display them on the cell surface to be noticed by the appropriate T cells. A large gene family encodes the MHC and MHC variants have a momentous impact on different biological traits of animals including susceptibility to infectious diseases, immune responses, the odor of an individual, mating preferences, pregnancy outcome and kin recognition. On the other hand, interferons, which are types of cytokines, refer to a class of signaling proteins that are generated and released by host cells in response to the presence of certain pathogens. These cytokines are not only responsible for increasing immunological responses but are also linked with various mechanisms to tackle the unrest caused by the foreign agents in the body. Interferons, once released, function to cause nearby cells to increase their anti-viral defenses. One mechanism through which interferons affect nearby cells is by causing the cells to up-regulate their antigen presenting ability by virtue of increasing the expression of MHC antigens. Once the antigen-presenting capability of these cells is heightened, they bind to the pathogens, breaking them down into their simpler components such as

---

peptides. This breakdown is catalyzed either through the endogenous or exogenous pathways of MHC presentation. Therefore, MHC molecules are a great area of study related to immunology and its role in the adaptation process by animals. This proves that MHC related genes make the best case for study of molecular adaptation in vertebrates (Sommer, 2005). The following study was designed and carried out not only to collect more genetic data for analysis on Pakistani camels, but to also sequence the immunity gene IFNβ1 of camel breeds from Pakistan for comparison with other species of animals to be able to make comments on genetic similarities and discrepancies, which in turn may aid our understanding of the resilience of the camel.

Materials and methods

DNA was extracted from blood samples through the inorganic method. Different blood samples from different camel breeds were used to analyze the immunological effect of IFNβ1. The specific primers i.e. IFNβ1-Fw CGGTGCCTCCTCCAGATGGTTC and IFNβ1-rev GGGGAACGATCGTGTCTTCCGT were designed by Primer3. Polymerase chain reaction (PCR) was used to amplify the end products of template DNA of camel breeds. The end products were then run on 0.8% agarose gel electrophoresis. Primers for Interferon Beta 1 (IFNβ1) were designed through Primer 3 software (Rozan and Skaletsky, 2000). The amplification of targeted regions (364 bp) was done through PCR using Bio-Rad C1000™ thermo cycler. The amplified PCR products were submitted for sequencing using Sanger sequencing method and these sequences were run on BLAST. The biological sequence alignment was done using BioEdit 7.0.9.0 (Hall, 1999). Sequence variation sites, nucleotide diversity and haplotype diversity were defined using DnaSP 5.1 software (Librado and Rozas, 2009). Lastly, phylogenetic analysis was carried out usingthe MEGA 6 program which showed the Phylogenetic lineage and ancestral history within the same species (Tamura *et al.*, 2011).

Results and discussion

Figure 1 shows the nucleotide sequence of IFNβ1 gene fragment (364 bp) aligned with reported sequences of different breeds of camels. Dual peaks/heterozygous conditions were identified at positions c.36 (A/C), c.87 and c.225 (Y, T/C).

The phylogenetic analysis of the IFNβ1 nucleotide sequences from different species using MEGA 6 showed clear separation of dromedary camel of Pakistan from other mammalian species. Our study analysis by neighbor joining method shows that there are five non-descript camel breeds due to no sequential breed information

phylogenetically and five Mareecha camel breeds which shows sequential relation to Mareecha breed as shown in (Fig. 2).
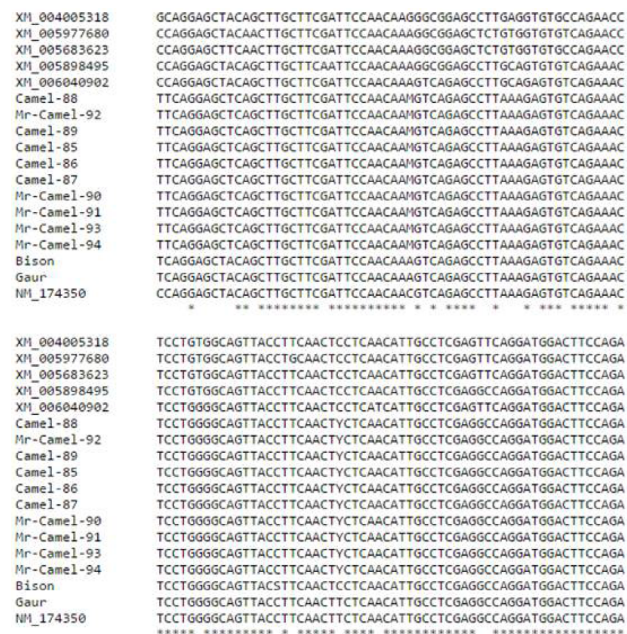

Fig. 1. Multiple Sequence alignment of IFNβ1 gene in dromedary camel of Pakistan and other mammalian species reported in GenBank
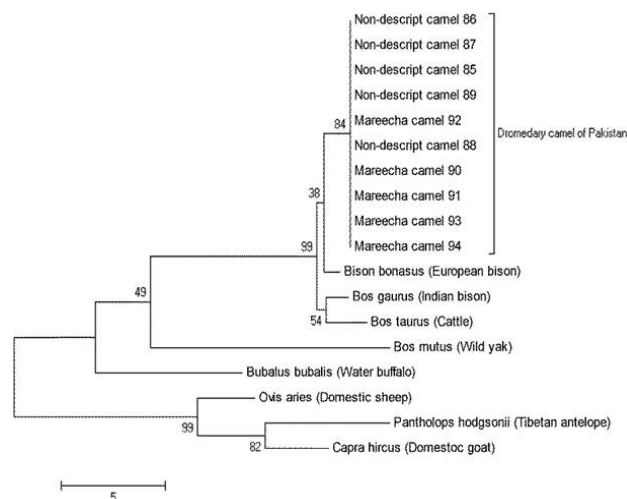

Fig. 2. NJ method based Phylogenetic tree depicting INFβ1 nucleotide sequences from different mammalian species in comparison to dromedary camel from Pakistan using MEGA6.

Camel's natural habitat is characterized as harsh conditions in the desert (Belkahia *et al.*, 2015). These

animals can bear fluctuation in body temperature and water loss for a relatively long time (Almathen *et al*., 2018). Despite the major importance of camels as locally adapted livestock in Pakistan, little information is available about the characterization of these economic animals (Tadese *et al*., 2019). There are several camel breeds that can be morphologically (especially based on coat color) identified in Pakistan (Khalkhali-Evrigh *et al*., 2018). Few studies are concerned with genetic variations between camel breeds in Pakistan at DNA level. This study aims to detect the molecular variations within and among some *Camelus dromedarius* for reconstructing the Neighbor-joining tree among applied camels. In addition, the study aims to explore the evolutionary variations between this species and other camelidae species based on some interferon beta gene sequences variations (Al-Atiyat *et al*., 2016). This tree was made using MEGA 6 software that showed the result in the form of a cladogram, depicting the lineage between different related species to the different dromedary camel breeds of Pakistan. The results were confirmed by constructing a gene tree and a species tree. In species tree, *Bos taurus* was nearest to *Bubalus bubalis* followed by *Ovis aries* thereby grouping the whole Bovidae family together. The gene tree constructed with the help of neighbor joining method clearly clustered closely related species in one clad.

Comparison of dromedary camel sequences from Pakistan were done according to similarity on the basis of genetic sequences of other animal species available on NCBI including Indian bison, European bison, domestic goat, Tibetan antelope, wild yak, sheep, water buffalo and cattle for biological positioning of dromedary camel. The results reconfirmed the classical biological classification. It is the first time reporting on IFNβ1 gene in dromedary Pakistani camels and hopefully it will pave the path for further studies on immunity genes to explore the camel's genetic worth.

Conflict of interest
The authors have declared no conflict of interest.

References

Ahmad, S., Yaqoob, M., Hashmi, N., Ahmad, S., Zaman, M.A. and Tariq, M., 2010. *Pak. Vet. J.,* **30**: 191-197.
Al-Atiyat, R.M., Suliman, G., AlSuhaibani, E., El-Waziry, A., Al-Owaimer, A. and Basmaeil, S., 2016. *Trop. Anim. Hlth. Prod*., **48**: 871-878. https://doi.org/10.1007/s11250-015-0990-5
Almathen, F., Elbir, H., Bahbahani, H., Mwacharo, J. and Hanotte, O., 2018. *J. Hered.*, **109**: 700-706. https://doi.org/10.1093/jhered/esy024
Al-Soudy, A., El-Sayed, A., El-Itriby, H.A. and Hussein, E.H.A., 2018. *J. Biodiv. Endang. Sp.,* **6**: 2.
Ali, R., Hussain, T., Babar, M.E., Masood, F., Ahmad, N., Pervez, M.T. and Nawaz, A., 2018. *Pak. J. agric. Sci.*, **55**: 2.
Belkahia, H., Said, M.B., Sayahi, L., Alberti, A. and Messadi, L., 2015. *J. Infect. Dev. Countr*., **9**: 1117-1125. https://doi.org/10.3855/jidc.6950
Hall, T.A., 1999. Nucl. Acids symp. Ser., **41**: 95-98.
Khalkhali-Evrigh, R., Hafezian, S.H., Hedayat-Evrigh, N., Farhadi, A. and Bakhtiarizadeh, M.R., 2018. *PLoS One,* **13**: 9, p.e0204028. https://doi.org/10.1371/journal.pone.0204028
Librado P. and Rozas J., 2009. *Bioinformatics*, **25**: 1451-1452. https://doi.org/10.1093/bioinformatics/btp187
Nazir, M.M., Oneeb, M., Ayaz, M.M., Bibi, F., Ahmad, A.N., Waheed, A., Sajid, M.A., Sultan, M.T., Yasin, G. and Lindsay, D.S., 2017. *Trop. Anim. Hlth. Prod.,* **49**: 1081-1084. https://doi.org/10.1007/s11250-017-1300-1
Rozen, S. and Skaletsky, H., 2000. *Bioinformatics methods protocols*, pp. 365-386. http://bioinfo.ut.ee/primer3-0.4.0/
Saad, Y.M., El Hanafy, A.A., Alkarim, S.A., Almehdar, H.A. and Redwan, E.M., 2017. *Int. J. Anim. Vet. Sci.,* **11***:* 564-568.
Sommer S., 2005. *Front. Zool.,* **2**: 1-18. https://doi.org/10.1186/1742-9994-2-16
Tadese, Y., Costa, V., Zelalem, R.M.G., Mengistu, N.A.A.U., Gebremeskel, M., Sisay, T., Kefelegn, K., Mohammed, Y.K., Solomon, A., Tadelle, D. and Beja-Pereira, A., 2019. *Ethiop. J. agric. Sci.,* **29**: 19-37.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. *Mol. Biol. Evolut.,* **28**: 2731-2739. https://doi.org/10.1093/molbev/msr121
Villela, A.D., Renard, G., Palma, M.S., Chies, J.M., Dalmora, S.L., Basso, L.A. and Santiago, D.S., 2010. *J. Microb. Biochem. Technol*., **2**: 111-117.
Vosoughi, R. and Marriott, J.J., 2014. *Mult. Scler. Relat. Disord*., **3**: 321-325.
Zidi, A., Sa`nchez, A., Ruff, G.O. and Amills, M., 2008. *J. Dairy Sci.,* **91**: 814-817. https://doi.org/10.3168/jds.2007-0342