RESEARCH ARTICLE

An Investigation on the Detection of Human Leucocyte Antigen HLA Class I Loci (A, B, C) and Class II Loci (DR, DQ) Allele Frequency in Nepalese Population by Next Generation Sequencing

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Abstract

Introduction: Human Leucocyte antigen (HLA) has offered a tremendous contribution to the human population by providing definite and undeniable facts of immense magnitude in human genetics, disease dynamics, and transfusion and transplantation.

Materials and Methods: Blood samples of 90 unrelated healthy populations residing in Kathmandu, a central region of Nepal, were collected. DNA was extracted from the blood samples, and the allele frequency for HLA class I loci (HLA –A,-B,-C) and II loci (DRB1 and DRQ1) was studied by ion torrent Next Generation Sequencing platform using GenDx NGSgo^R workflow. Further, comparing the most frequently detected HLA alleles in the Nepalese population with populations of neighboring countries was also done.

Results: A total of 10 HLA *A alleles, 18 HLA*B alleles,11 HLA*C alleles,11 HLA*DRB1, and 4 HLA*DQB1 were detected. The most common alleles detected were HLA A*01(16.67%), A *33(31.67%), HLA B*35(13.33%) B*44(11.67%) HLA C* 04(16.67%),C* 07(23.33%), C*15(16.67%).HLA - DR*07(16.67%),DR*15(25.0%) HLA-DQ *05(38.33%) respectively. Comparison with a population of the neighboring countries and Caucasian population revealed that these common alleles were also present in high frequency in North Indian Hindus and some frequencies with Mongolian and Caucasian population but not with the Chinese population.

Discussion: We believe that this data is the first report of HLA class I loci (HLA A, B, C) and II loci (DR*B1 and DQ*B1) in a healthy population from Nepal, and this provides helpful information with diverse applications in Nepal.

Keywords: HLA, Healthy population, Nepal, Infectious disease, Next Generation Sequencing

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Introduction

Nepal, an Asian landlocked country, is located between China to the north and India to the east, west, and south. It has a territory that extends roughly 90 to 150 miles from north to south and 500 miles from east to west.[1] Despite its size, the country has a prodigious geographic diversity covering as low as 59 meters and covers as high as 8848 meters. The country is divided mainly into 3 belts which are Terai, Pahad, and Himal.[2] Furthermore, Nepal is a cultural mosaic comprising Tibeto-Burman and Indo –Aryan linguistic families, which is the consequence of the migrations from east, west, north, and south respectively over 2000 years.[3,4] The Indo-Aryan family constitute Nepali, Maithili, Bhojpuri, and Tharu, whereas Tibeto Burman families included Tamang, Newari, Magar, Rai-kiranti, Gurung, and Limbu.[3,5]

Kathmandu metropolitan is the capital city of Nepal and has played a central role in representing the cultural, caste/ethnic mosaic of the nation. It historically represents Newar settlement but has also witnessed a high influx of population due to migration in the city.[6,7] There are reports that among other castes/ethnic groups present in Nepal, the newars, brahmins, chhertri, and tamang are the most predominant casts/ethnic group present in Kathmandu.(8) Thus, from the above, Nepal has a heterogeneous group of population.

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Known to be highly polymorphic, closely linked genes that can split into many allelic types, the human leukocyte antigen (HLA) holds immense importance in physiological and pathological conditions. The HLA complex is considered a potent marker of population genetic analysis, paternity determination, and various disease-associated studies.[9-12]

Besides this, the HLA profile has played a key role in the selection of donors for successful transplantation and transfusion.[13]

Minimal data on HLA profiling of Nepali population in some conditions along with worldwide data have been documented previously.[14] However, there are early reports of Nepali population migration to India, mostly known as Gorkhas[15-17] but very scarce information is available regarding HLA typing in such population.[18] However, the report on the healthy population residing in Nepal is yet to be done.

In the present investigation, the frequency of HLA A1, A2, B1, B2C1, C2, DR1, and DQ1was studied in a healthy Nepali population from Kathmandu city, and it was further compared with the neighboring countries of Nepal and the Caucasian population to understand the frequency of prevalence of the HLA types and genetic diversity in Nepalese population present in Kathmandu valley.

Methods And Materials

The below-mentioned work is a preliminary pilot study conducted on the healthy control population, a part of the HLA class I and II work done in hepatitis B positive patients.

Ethical approval

The ethical approval was taken by the ethical committee of the Review Board of Nepal, The Nepal health research council (Ref Number:138/2018). Furthermore, written informed consent was taken from all the study participants before commencing the study.

Characteristics of Healthy Subjects

This was a pilot study in which 90 healthy individuals, 60 males and 30 females (30.75+-8.59), were recruited, corresponding to 90 Hepatitis B infected patients. The healthy individuals were negative for the serological markers of Hepatitis B virus infection (HBV), HIV, and Hepatitis C virus infection (HCV). None of the participants were related to each other.

DNA Extraction and HLA Typing

According to the manufacturer's instructions, the DNA was extracted from the stored blood samples using the QI amp DNA mini kit(Qiagen, Hilden, Germany). Then, extracted DNA was quantified by NanoDrop (Thermo Scientific, USA).

The extracted and quantified DNA was then sent to Supratech Laboratories Pvt. Limited, Ahmadabad, India, to type class I and class II HLA molecules. This included HLA A, HLA B, HLA C, HLA DR, and HLA DQ. Finally, the amplification of each DNA sample was carried out by Next-Generation sequencing (NGS) with the Ion Torrent NGS platform using GenDx NGSgo^{R} workflow.

Statistical Data Analysis

The HLA allele frequency of classes I and II was calculated and then compared with the chi-square test and/or Fishers exact test. Further, Statistical analysis was performed by IBM SPSS statistic version 20.

Results

the HLA –A, B, C, DR, and DQ frequencies in the Nepalese population from Kathmandu have been represented in tables 1A,1B,1C,1D,1E, and figures 1,2,3,4, and 5 respectively.

HLA alleles A*01 and A*33 are the most frequent allele in Nepalese population.

The DNA was isolated from the collected blood in EDTA vials of study participants. The purity and concentration of isolated DNA were evaluated on nanodrop. The good quality of DNA obtained (A_{260}/A_{280} = 1.7 to 2.0) was used for sequencing of Class-I (HLA-A, B, C) and Class-II (HLA-DRB and HLA-DQB) alleles. A total of 10 different HLA-A alleles have been detected. The sequencing data showed that alleles A*01(16.67%) and A*33(31.67) were detected in high frequency in healthy individuals, followed by A*02(13.33%) and A*24(13.33%) (Table1A, Figure1). The diversity obtained being A*01:01:01 and A*33:03:01. Regarding A*02, the diversity obtained being 02:01:01,02:05:01,02:06:01,02:11:01 and 24:02:01.

Table 1A: The table denotes the frequency of the HLA *A alleles in Nepalese population.

A allele	*Healthy frequency(n = 90)*
A*01	16.67%
A*02	13.33%
A*03	5.00%
A*11	6.67%
A*24	13.33%
A*26	1.67%
A*29	8.33%
A*31	1.67%
A*32	-
A*33	31.67%
A*34	-
A*68	1.67%

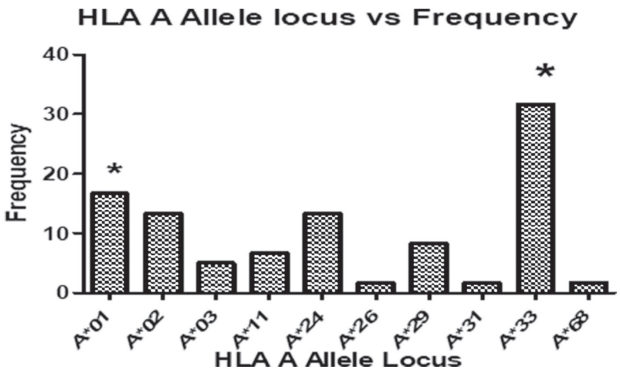

Figure 1: The figure denotes the most frequent HLA *A alleles (HLA A*01 and A*68) in Nepalese population.

HLA alleles B*44 and B*35 are the most Frequent alleles in the Nepalese population.

Regarding B Locus, 18 different alleles have been observed. The most frequent alleles being B*44(11.67%) and B*35(13.33%) (Table 1B, Figure 2). The diversity detected being B*44:03:02 and B*35:01:01,35:03:01 respectively.

Table 1B: The table denotes the frequency of the HLA *B alleles in Nepalese population.

*HLA*B Allele*	*Frequency (n = 90)*
B*07	8.33%
B*08	-
B*13	-
B*15	6.67%
B*18	1.67%
B*27	6.67%
B*35	13.33%
B*37	1.67%
B*38	3.33%
B*39	1.67%
B*40	3.33%
B*41	-
B*44	11.67%
B*48	1.67%
B*50	1.67%
B*51	6.67%
B*52	5.00%
B*55	8.33%
B*56	1.67%
B*57	6.67%
B*58	10.00%

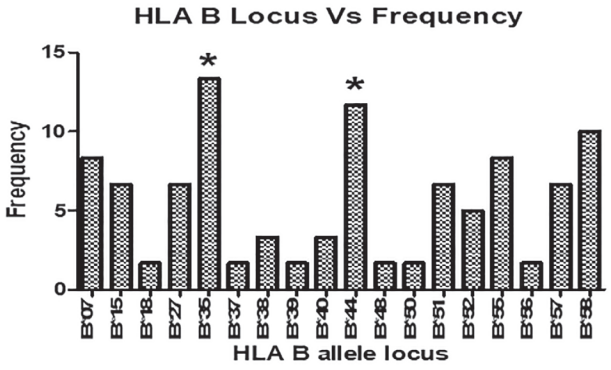

Figure 2: The figure denotes the most frequent HLA *B alleles(HLA B*35 and B*44) in Nepalese population.

HLA alleles C*07, C*15, and C*04 are the most frequent alleles in the Nepalese population.

Regarding C locus, 11 different alleles have been detected. The most frequent alleles being C*07(23.33%),C*15(16.67%) and C*04(16.67%) followed by C*01(11.67%)(Table 1C,Figure 3). The diversity detected being 04:01:01:01,04:01:01:06, 07:01:01:01, 07:01:01:04, 07:01:02:01, 07:02:01:01, 07:02:01:03 and07:06:01:01.Further the diversity detected was 15:02:01:01, 15:05:01:01, 15:05:02:01 and C*01:02:01:01 respectively.

Table 1C: The table denotes the frequency of the HLA *C alleles in Nepalese population.

HLA Allele C	*Frequency (n = 90)*
C*01	11.67%
C*02	1.67%
C*03	10.00%
C*04	16.67%
C*05	-
C*06	6.67%
C*07	23.33%
C*08	1.67%
C*12	5.00%
C*14	3.33%
C*15	16.67%
C*16	3.33%
C*17	-

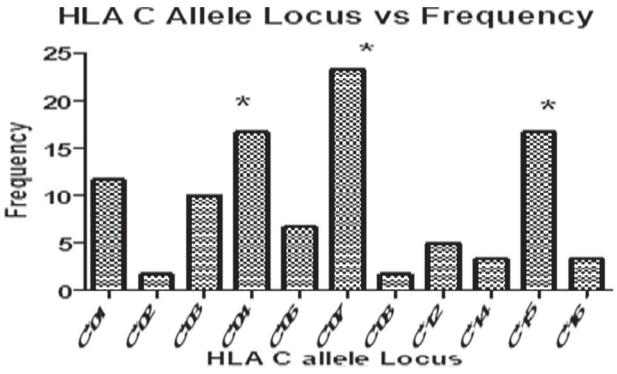

Figure 3: The figure denotes the most frequent HLA *C alleles (HLA C*04,07 and 15) in Nepalese population.

HLA alleles DR*15, DR*07, and DQ*05 are the most frequent alleles in the Nepalese population.

Regarding DR and DQ alles, 11DR and 4DQ alleles were detected respectively. The most frequent alleles being DR*15(25%), DR*07(16.67%) and DQ*05(38.33%) (Table 1D, Figure 4, and Table 1E, Figure 5 respectively).The diversity detected being DR*15:02:01:01,15:01:01:01, DR*15:04, and DR*07:01:01:01. Diversity of DQ*05:02:01:01, 05:02:01:02, and 05:03:01:01 was detected.

Table 1D: The table denotes the frequency of the HLA *DR alleles in Nepalese population.

AlleleDR	*Frequency(n = 90)*
DR*01	5.00%
DR*02	1.67%
DR*03	6.67%
DR*04	6.67%
DR*07	16.67%
DR*08	-
DR*09	-
DR*10	11.67%
DR*11	3.33%
DR*12	10.00%
DR*13	1.67%
DR*14	11.67%
DR*15	25.00%
DR*16	-

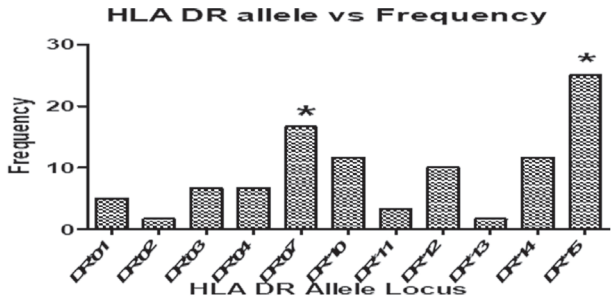

Figure 4: The figure denotes the most frequent HLA *DR alleles (HLA C*07 and 15) in Nepalese population.

Table 1E: The table denotes the frequency of the HLA *DQ alleles in Nepalese population.

HLA Allele DQ	*Frequency(n=90)*
DQ*02	18.33%
DQ*03	25.00%
DQ*04	-
DQ*05	38.33%
DQ*06	18.33%

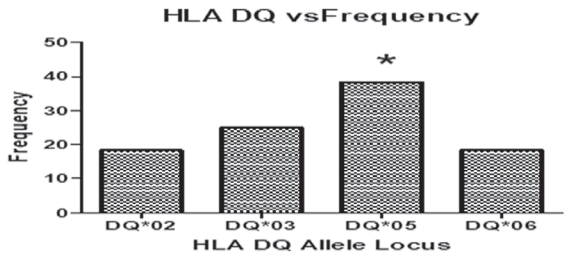

Figure 5: The figure denotes the most frequent HLA *DQ alleles (HLA DQ*05) in Nepalese population.

Discussion

To the best of our knowledge, this study is the first study of an eight-digit HLA study of both HLA classes I and II in the Nepalese population from Nepal. However, only two digit data analysis was performed to prevent biasness in results due to fewer sample sizes. HLA has been known to play a major role in the genetic diversity of a population and organ transplantation, its association with infectious disease, in understanding drug reactions, and its relevance to adaptive immunity and vaccine development.

Regarding allele A, a comparative study with the neighboring countries shows a high frequency of A*0101 in the north Indian population.[19,20] Further, A*3303 is also reported to be high in Asia and the Japanese and Korean populations, indicating their origin from the Mongolian population or the Mongolian pool.[18,21-26] A*33 have also been reported to show greater diversity with the existence of several unique alleles.[27] However much more detailed work has to be done in the Nepali population in this regard. HLA A*24 is also one of the frequent alleles similar to the north Indian population.[28] A*0101 was not detected in an earlier study in the Nepalese population.[32] The absence or presence of such allele could be due to the precise use of the Next Generation Sequencing technique used in our study. Our investigation also shows the presence of allele A*0211, A*0206, and B*2705 in the Nepalese population.These have also been found to be the frequent alleles found in Asian Indians.[42,43]

The data is further compared with china (Tibet), the neighboring country of Nepal. The most frequent allele is A*110101/1121N in the Chinese population, which is detected in lower Nepali populations. However, HLA*0101 is reported as an allele of high frequency in Caucasians and Jews and is often found oftenly in Nepali populations.[29] Besides this, the Caucasian population shows a high frequency of alleles A 150101(32.9%) and 070101 (30.1%), which is very different from the observations available here in the Nepali population.

Regarding allele B, The allele B*44 and B*35 are also most common alleles in North Indian population and Gurkha population.[18,30,31] Some of these alleles have also been reported previously from a Renal transplant study conducted in Nepal[32] and found to be the frequent alleles in the Caucasian population.[33,34] Our study reveals the prevalence of the allele HLA B*3503 in high frequency. The allele, as mentioned above, has also been associated with the progression to AIDS.[40] There are reports that HLA B*3503 makes the population more susceptible to the rapid progression to AIDS after infection.[19] Regarding HLA C, the common alleles found were C*04(16.67%), C*07(23.33%), and C*15(16.67%). Of all these, Cw 04 is one of the most common HLA C alleles reported in all population.[29]

Regarding the allele DR, our comparison with other neighboring countries of Nepal revealed that the allele DR*1501 was common in the North Indian population but is infrequently found in the Tibet region of China, which is

on the close border with Nepal. Also, DR*1501 are common alleles in Asia as well as in Uttar Pradesh, a region that lies in India and the close border with Nepal.[35,36]

Regarding allele DQ, DQ*05(38.33%) was the most frequently detected allele in the Nepalese population. DQ0301 and DQ0501 have also been reported frequently in the North Indian populations and caucasain populations, respectively.[37-39] Similarly, DQB1*0301 alleles have been associated with persistent Hepatitis B infection in African Americans.[41] Prevalence of this allele in high frequency in a Healthy population can be an alert sign for the individuals to be cautious. However, more extensive data is needed to for the best implication of its application.

Conclusion

The HLA data for the healthy population of Nepal shows a variety of heterogeneity. However, the data regarding HLA from Nepal and the countries neighboring Nepal is limited, and there is a strong need to type the HLA loci of these populations to understand better the role of HLA and its effect on the diverse population. Therefore, the paper under discussion will provide information on the genetic diversity prevalent in Nepal's Kathmandu valley.

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