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ORIGINAL ARTICLE

Single Nucleotide Polymorphisms (SNPs), Phylogeny and Spatiotemporal analysis of pro gene in HIV samples from Pakistan

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ABSTRACT

Background: The increase in incidence of HIV infection with limited treatment options has enhanced the morbidity and mortality in South Asian countries. The mutations, geographical and genetic diversity in Pro gene in HIV positive patients from other South Asian countries can be helpful for treatment options.
Aim: To identify mutations and genetic diversity in Pro gene in HIV positive patients in Pakistan and compare the sequences to neighbouring countries China and India.
Methods: The HIV protease gene or pro-gene sequence were retrieved from NCBI. The sequence were studied from Pakistan, China and India were analysed for their linkage and genetic mutations through bioinformatics tools, MEGAX and CLUSTALW
Results: The phylogenetic analysis of samples for maximum likelihood and mutation from South Asian countries Pakistan, China and India individually displayed variation in sequences of HIV pro gene in all these isolates. Whereas Pakistani isolates have more genetic similarities with the isolates from China than India.
Conclusion: The phylogeny analysis depicts there is gradual evolution in viral types and possible entry is through the neighbouring transmission might have been through social connections. The mutations studies provides bases for the novel targets for the drug design and development against HIV.
Keywords: HIV, Protease gene, MEGA, Phylogenetic analysis, Pakistan

INTRODUCTION

Acquired immunodeficiency syndrome AIDS being potential life threatening disease its severity is due the human immunodeficiency virus HIV[1]. The human immunodeficiency viral disease has challenged the human race worldwide it mainly affects the immune system of the AID's patient. Pakistan in South Asian countries is stated as low prevalent but high risk, since the year 2000 till now almost 220000 positive HIV cases aged 15-50 years have been reported[2]. The two major strains of human immunodeficiency virus are HIV-1 and HIV-2 where the strain I has be more frequent and virulent however the = strain II has lower infectivity documented[3]. There are different modes of transmission for this blood borne virus which includes, heterosexual contact, perinatal transmission transfer of virus from mother to foetus and through in a needle prick[4]. Once acquired immunodeficiency syndrome AIDS affects the body it suppresses the immune system and makes body susceptible for several opportunistic infections which causes severe illness[5]. This study is based on the analysis of SNPs the single nucleotide polymorphism by analysing the pro gene of the HIV genome from Pakistan, China and India the neighbouring countries. This structural gene has 297 base pairs of nucleotide sequence and the protease enzyme which encodes by the Pro gene is aspartic protease. In this study, the single nucleotide polymorphisms (SNPs), mutations and genetic variations in a pro gene in South Asian countries Pakistan, China and India have been analysed using bioinformatics tools.

MATERIAL AND METHODS

Sequence Retrieval for Protease gene from South Asian countries: After approval from LMDC Ethical Committee, the protease gene sequences for "pro-gene" were retrieved from National Centre for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/. The protease gene sequences were retried for Pakistan, China and India the South Asian countries number of sequences retried from each of the three countries were 174, 117 and 105 respectively. The pro-gene nucleotide sequences were downloaded in the text based sequence format "FASTA" for further analysis of 297 nucleotides of pro gene as given:
TGTACTGAGAGACATGGCTAATTTTTTAGGGAAGATCT
GGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCA
GAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAG
CTTCAGGTCTGGGGTAGAGACAACAACTCCCCCTCAG
AAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAAC
TTC**CCTCAGGTCACTCTTTGGCAACGACCCCTCGTCA**
CAATAAAGATAGGGGGGCAACTAAAGGAAGCTCTAT
TAGATACAGGAGCAGATGATACAGTATTAGAAGAAAT
GAGTTTGCCAGGAAGATGGAAACCAAAAATGATAGG
GGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGAT
CAGATACTCATAGAAATCTGTGGACATAAAGCTATAG
GTACAGTATTAGTAGGACCTACACCTGTCAACATAAT
TGGAAGAAATCTGTTGACTCAGATTGGTTGCACTTTA
AATTTTCCCATTAGCCCTATTGAGACTGTACCAGTAAA

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ATTAAAGCCAGGAATGGATGGCCCAAAAGTTAAACAAT
GGCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAA
ATTTGTACAGAGATGGAAAAGGAAGGGAAAATTTCAAA
AATTGGGCCTGAAAATCCATACAATACTCCAGTATTTG
CCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTA
GTAGATTTCAGAGAACTTAATAAGAGAACTCAAGACTT
CTGGGAAGTTCAATTAGGAATACCA

Sequence Alignment: The protease gene sequences downloaded for each of three selected countries Pakistan, China and India the sequences were aligned by Molecular Evolutionary Genetics Analysis MEGAX online server, https://www.megasoftware.net. The MEGAX is an integrated Bioinformatics tool sequence alignment, deducing phylogenetic trees, assessing divergence, mining databases, approximating molecular evolution and testing ancestral sequences for evolutionary information. MEGAX is for evaluating the reconstructing of evolutionary histories of different species by selecting nucleotide or protein sequences. The similarities and diversification among the protease sequences from selected South Asian countries were examined by Multiple Sequence Alignment for protein or DNA https://www.genome.jp, Clustalw http://www.ebi.ac.uk/Tools/msa/clustalw2/.

Phylogenetic Analysis of protease gene: The Bioinformatics approach for reconstruction of phylogeny with more recent historically nested with more distant ancestors through to interpreted the relation between organisms is Cladistics. Therefore the investigation for nested groups searching for shared derived character states, the clades the monophyletic groups MEGAX was utilized for maximum parsimony, neighbour joining, and distance-based on phylogenetic tree besides Crustalw multiple sequence alignment function for molecular evolution estimation.

RESULTS

The pro gene sequence retrieved National Center for Biotechnology Information (NCBI) of protease gene from Pakistan, China and India was aligned through MEGAX the variable and conserved regions were observed in the sequences selected. In Medical Sciences research phylogenetic analysis has are vital for origin along with reconstruction of geographical linkages and time of divergence.

The Phylogenetic analysis of South Asian countries selected Pakistan, China, and India was accomplished to detect the discrepancy or variations in pro gene of HIV in the population of neighbouring countries of Pakistan. The individual as well as collective Phylogenetic analysis of these countries shows that there is extreme variation among the sequences selected from China being most populated country of the world there is increased number of mutations with the time (Figure 1). However, on contrary to China, India another most populous country has minimum variations, this may be due to the century's old conserved social system of India (Figure 2).

Although for Pakistan, interestingly there were some researches that studies the pro gene sequence in some regions has variations, but of the region of the pro gene was conserved. Moreover, the while analysing the phylogenetic tree of Pakistan with its close neighbours China and India the similarity with lying the branches near Chinese analysis was observed much more than that of Indian sequences (Figure 3). The reason for this can be closed boarders with India has prevented the viral transmission and travelling, trading and studying China has been one of nearest destination for people of Pakistan.

DISCUSSION

The Acquired immunodeficiency syndrome (AIDS) human immunodeficiency virus HIV has the ability to affect and damage the pivotal cells of human immune system. There are variable factors which are responsible for the genetic evolution of a virus including mutation, selection forces, random drift, recombination and transmission among the individuals. The HIV Protease gene sequence from South Asian countries comprising of Pakistan, China and India to investigate the **s** origin and spatiotemporal affect in Pakistan[6]. The Protease gene sequence of Pakistan was matched with both countries which shows the alignment of sequences from these countries individually as well as comparatively indicates the conserve and variable regions[7].

Furthermore, there were enhanced variations were observed for Chines sequences ,while, in India, there were less variation recorded[8]. The isolates from Pakistan in phytogenic relationship were closely associated to Chinese isolates this predicts that the virus may been transmitted to Pakistan as there is frequent travelling from Pakistan for trade and study[9]. The HIV protease gene variations may be the reason for mutations that occurred in the genome of the virus spatiotemporal and with the evolution. Moreover, these can be deletion, insertion or substitution mutations[10]. The relationship between drugs (heroin) and the spread of HIV among IDUs the injecting drug and their sex companions in South Asian countries has been considered foremost route for the disease transmission. HIV modes of transmission in Pakistan are dominated by heterosexual reported approximate rate of 52.55%[11], the contaminated blood 11.73%[12] and IDUs is 2.02%[13]. The protease enzyme which is encoded by the Pro gene is an aspartyl protease that has a function as a dimer for protease activity and cleavage of Gag and Gag-Pol polyprotein precursors during virion maturation.

The Phylogenetic information observed and documented by this research can be used to the reconstruction of the evolutionary history and design and develop targeted therapeutic candidate drugs by inhibiting the mutated pro genes to control AIDS.

CONCLUSION

The sequences of protease gene from South Asian countries Pakistan, China and India have analysed the phylogenetic analysis showed that there is high number variation in sequences in case of China, however, is less variation in case of India. The isolate from Pakistan were more related to China which may be due bulk travelling, trading, and study purpose tour from Pakistan. The bioinformational analysis to study the association of mutations in pro genes isolated from Pakistan AIDS patients. And also information provided by this research can be used to make new targeted drugs and anti-viral therapy to control AIDS by inhibiting the mutated proteins.

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Fig. 1: China, protease gene phylogenetic analysis of isolates

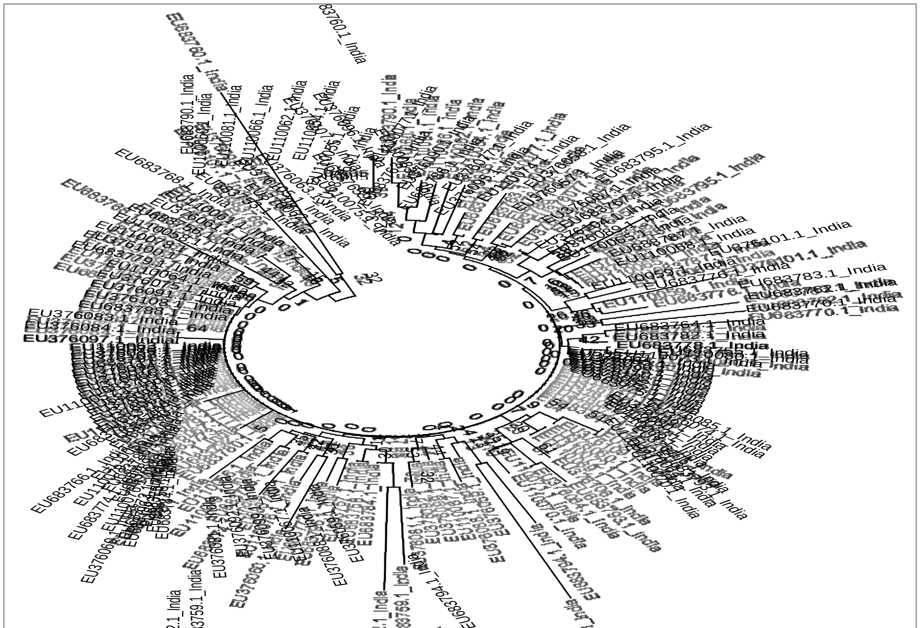

Figure 2: India, protease gene phylogenetic analysis of isolates

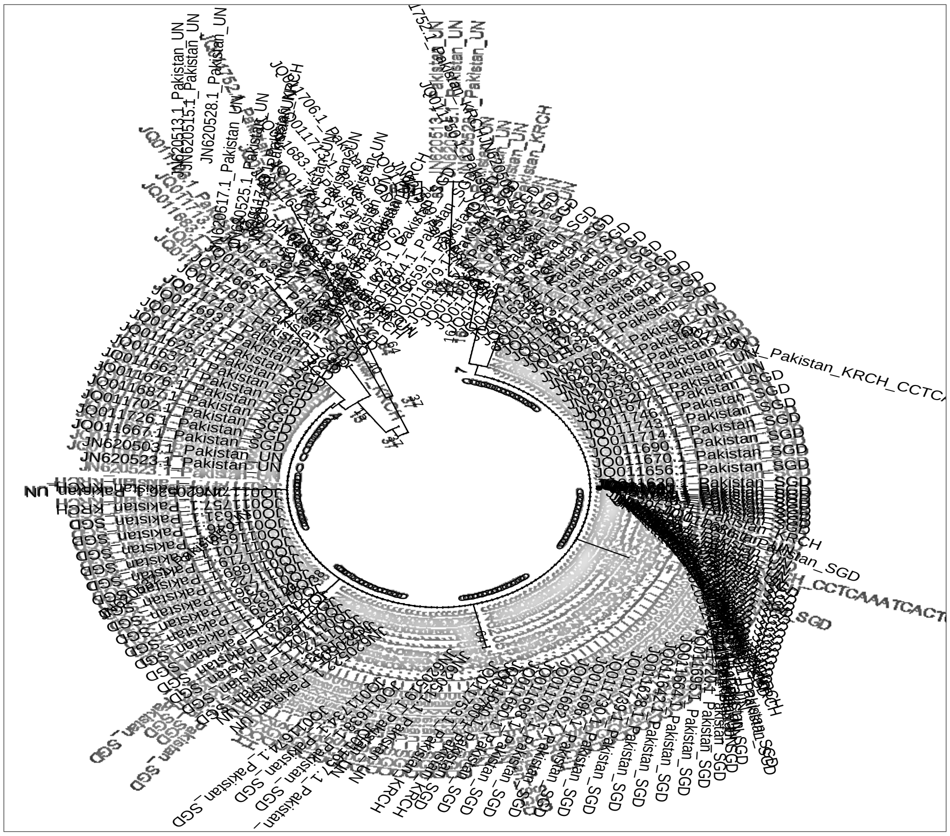

Figure 3: Pakistan, protease gene phylogenetic analysis of isolates