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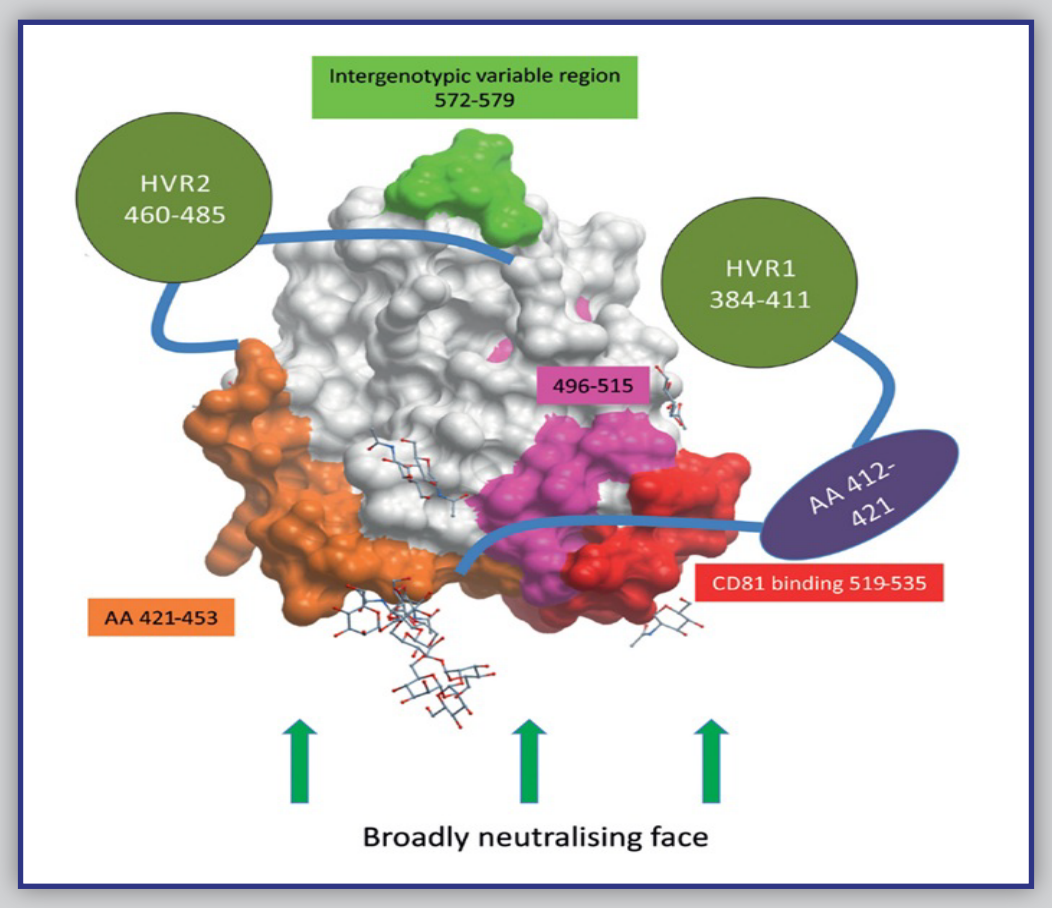

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PMDC NO 20, 1989. INDEXED ON CPSP MEDILINE

“HCV ENVELOPE GLYCOPROTEIN 2 SURFACE REPRESENTATION”

E2 is a globular protein with three regions of hypervariablity - HVR1, HVR2, and intergenotypic variable region - shown in green. HVR1 is predicted to mask a hydrophobic region that is sensitive to neutralizing Antibodies (nAbs).
E2’s broadly neutralizing face, where many broadly neutralizing Abs bind, comprises CD81-binding loop (in red), residues 421-453 (in orange), residues 502-520 (in pink), and residues 412-421 (in purple). The possible positions of some glycans are shown as stick and ball figures.

Courtesy:
Kong L, Giang E, Nieusma T, Kadam RU, Cogburn KE, Hua Y, et al. Hepatitis C virus E2 envelope glycoprotein core structure. Science (2013) 342:1090-4.

وَأَمَّا مَا يَنْفَعُ النَّاسَ فَيَمْكُثُ فِي الْأَرْضِ ۚ

اور جو لوگوں کو فائدہ پہنچاتا ہے، وہ روئے زمین پر قائم رہتا ہے۔ (الرعد : ۱۷)

Which is for the good of mankind remains in the earth

The Pakistan Journal *of* GASTROENTEROLGY

For Correspondence: Executive Editor

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TEAR HERE

HEPATITIS C VIRUS VACCINE; WHY STILL IS A HOPE?

Hepatitis C Virus (HCV) infections are usually asymptomatic before the beginning of advanced liver disease. Similarly screening for HCV is generally not performed in majority part of the world; hence HCV infection is not timely identified in most people.[1] Even after identification every patient cannot get treatment due to the lack of free availability and the cost of treatment.

Although the oral direct-acting antivirals (DAAs) eradicate HCV infection, yet numerous limitations of treatment necessitate a preventive vaccine necessary to take it under control just like polio. Some treated patients have evolved resistance even to DAAs, which is likely to increase further after free availability of these drugs to masses.[2] Liver disease can grow to cancer despite cure of the HCV infection in cirrhotic patients as treatment does not eradicate all of the complications of HCV infection, therefore prevention of chronic infection gives a great advantage over treatment.

Even with more than 95% cure rates with DAA, HCV eradication is still difficult due to reinfection, as immunity after curative treatment is not able to escape persons from reinfection of HCV especially in individuals with high risk like men having sex with men, intravenous drug abusers, and workers in health care facilities who are exposed to infectious fluids and blood products.[3-6]

Global control requires consistently and significantly higher annual rates of cure than new HCV infection rates by 2030.[7-8] An effective preventive vaccine will have a noteworthy effect on HCV occurrence and of great help to achieve HCV control globally. However, there are barriers to develop a vaccine, including diversity of virus, restrictions to HCV culture systems, limited models, incomplete knowledge of protective immune responses, and risk to people for testing vaccines.

The inactivated whole virus and live-attenuated vaccines are very efficient against other viruses; however, incapability to culture HCV has made very difficult to produce an inactivated whole HCV virus or live-attenuated HCV vaccine.[9] Due to adaptive mutations in HCV culture, virus has increases replication in vitro, while in nonhuman primate cell lines, it does not replicate rapidly, and virulence factors for hepatitis C virus have also not been clearly understood and defined. Extraordinary genetic diversity of HCV is a big challenge for the development of the vaccine. Seven genotypes and more than eighty subtypes, and error-prone polymerase of HCV and immune choice generate a diverse quasispecies of HCV variants within each infected person, creating many chances for selection of HCV variants with resistance to antibody and T-cell responses.[10-16]

The finding of a new HCV-like virus, the rat Hepacivirus (but limited sequence homology with HCV), will make a small animal model for vaccine testing.[17-18] An effective vaccine is difficult to legalize unless it is tested in peoples with a more chance for HCV infection and vaccinating them before HCV exposure. Knowing what immune responses show protective immunity would allow testing of vaccines in healthy individuals not at risk for infection first. On average, 25% of infected persons clear HCV spontaneously.[19] Furthermore, reinfections are cleared more frequently than primary infections and are linked with broadened cellular adaptive immune responses and the existence of broadly cross-reactive neutralizing antibodies (NAbs).[20-21]

It has been discovered in research that HCV-specific CD8+ cytotoxic T cells, CD4+ helper T cells, and antibodies all play a part in defense against persistent HCV infection. Broadly directed HCVspecific CD4+ T cells are detectable during early course of HCV infection but become functionally defective during chronic phase of infection, and then rapidly become undetectable, impair the response of CD8+ T cells, and, results T-cell exhaustion, and escape mutations in class I epitopes of HCV variants.[22-23] A successful vaccination requires an effective memory response. However, in case of HCV, lacking of memory cells required for protective immunity, and escape of viral persistence following primary infection has made difficult the emergence of effective HCV vaccine.

The sterilizing immunity induced by the efficient vaccines will prevent infection persistence and will decrease HCV related mortality and morbidity without lowering the incidence of new infection. A reduced level of viremia during acute viral phase with a vaccine would also be beneficent in term of less HCV transmission rate due to low residual titers of infectious virus in blood.[24-25] Another method is to target the nonstructural proteins of the genome (NS3, NS4, and NS5) to induce a broad T-cell response, using simian adenoviral vectors to target multiple HCV genotypes.[26-27] Some vaccines have been evaluated for immunogenicity and capability to shelter the chimpanzees from HCV include Virus like recombinant nonstructural proteins, Protein (VLP) containing the HCV E1, E2, and core proteins; framed with the ISCOMATRIX adjuvant; and genetic vaccines encoding nonstructural

proteins.[28-32] When the chimpanzees were immunized with recombinant NS3, NS4, and NS5 proteins, they resulted in persistent HCV infection on rechallenge, but when naïve chimpanzees were vaccinated with modified recombinant vaccinia (MVA)-having core-E1-E2 and NS3 genes as a boost, and DNA plasmids harboring the core-E1-E2 and NS3 for priming, they attained HCV-specific antibody and T-cell responses.[33-34]

Two vaccines (Ad6-NS and ChAd3-NS), which prevent HCV infection by provoking T-cell intermediated immunity have been checked in phase I (safety and immunogenicity) trials in human volunteers not at risk for HCV infection, were well tolerated and highly immunogenic.[35-37] The MVA-NS boost and ChAd3-NS prime stratagem is being assessed in a staged phase 1/2 study, with primary endpoint of preventing HCV persistence in HCV-naïve populations of PWID at high risk for infection but further trails will be required for confirmation. HCV clearance is associated with the early production of broadly neutralizing antibodies (bNAbs) resulting in spontaneous clearance of HCV infection.[38-41] Overall, more work is required to recognize optimal vaccine antigens, as well as ideal vaccine adjuvants.

The knowing of the mechanisms, by which antigen-specific immune cells facilitate long-term protection and decrease the vast diversity of HCV must produce a broad immune response, capable of responding to numerous variations are the major goals. Phase 1 studies are in pipline to evaluate healthy volunteers, with outcomes probable in the current year. Effective control of HCV infection will most likely require a combination of large-scale screening to identify infected persons, treatment of infected peoples, and harm-reduction and preventive strategies for those individuals who are uninfected and are at the risk. A prophylactic HCV vaccine is an important part of a fruitful plan for global control, though its creation is not easy.

REFERENCES

1. 1. GravitzL. Introduction: a smouldering public-health crisis. Nature. 2011;474:S2–S4.
2. 2. Franco S., Tural C., Nevot M. Detection of a sexually transmitted hepatitis C virus protease inhibitorresistance variant in a human immunodeficiency virus-infected homosexual man. Gastroenterology. 2014;147:599–601.e1.
3. 3. Midgard H., Bjoro B., Maeland A. Hepatitis C reinfection after sustained virological response. J Hepatol. 2016;64:1020–1026
4. 4. Martin T.C., Martin N.K., Hickman M. Hepatitis C virus reinfection incidence and treatment outcome among HIV-positive MSM. AIDS. 2013;27:2551–2557.
5. 5. Pineda J.A., Nunez-Torres R., Tellez F. Hepatitis C virus reinfection after sustained virological response in HIV-infected patients with chronic hepatitis C. J Infect. 2015;71:571–577.
6. 6. Martinello M., Grebely J., Petoumenos K. HCV reinfection incidence among individuals treated for recent infection. J Viral Hepat. 2017;24:359–370.
7. 7. Hill A.M., Nath S., Simmons B. The road to elimination of hepatitis C: analysis of cures versus new infections in 91 countries. J Virus Erad. 2017;3:117–1238.
8. 8. World Health Organization Global Hepatitis Report 2017.
9. 9. Thomas E., Liang T.J. Experimental models of hepatitis B and C—new insights and progress. Nat Rev GastroenterolHepatol. 2016;13:362–374.
10. 10. Martell M., Esteban J.I., Quer J. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. J Virol. 1992;66:3225–3229.
11. 11. Farci P., Bukh J., Purcell R.H. The quasispecies of hepatitis C virus and the host immune response. Springer SeminImmunopathol. 1997;19:5–26.
12. 12. Forns X., Purcell R.H., Bukh J. Quasispecies in viral persistence and pathogenesis of hepatitis C virus. Trends Microbiol. 1999;7:402–410.
13. 13. Cox A.L., Mosbruger T., Mao Q. Cellular immune selection with hepatitis C virus persistence in humans. J Exp Med. 2005;201:1741–1752.
14. 14. Erickson A.L., Houghton M., Choo Q.L. Hepatitis C virus-specific CTL responses in the liver of chimpanzees with acute and chronic hepatitis C. J Immunol. 1993;151:4189–4199.
15. 15. Liu L., Fisher B.E., Dowd K.A. Acceleration of hepatitis C virus envelope evolution in humans is consistent with progressive humoral immune selection during the transition from acute to chronic infection. J Virol. 2010;84:5067–5077.
16. 16. Timm J., Lauer G.M., Kavanagh D.G. CD8 epitope escape and reversion in acute HCV infection. J Exp Med. 2004;200:1593–1604.
17. 17. Billerbeck E., Wolfisberg R., Fahnoe U. Mouse models of acute and chronic hepacivirus infection. Science. 2017;357:204–208.
18. 18. Trivedi S., Murthy S., Sharma H. Viral persistence, liver disease and host response in Hepatitis C-like virus rat model. Hepatology. 2017;68:435–448.
19. 19. Micallef J.M., Kaldor J.M., Dore G.J. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat. 2006;13:34–41.
20. 20. Osburn W.O., Fisher B.E., Dowd K.A. Spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection. Gastroenterology. 2010;138:315–324.
21. 21. Sacks-Davis R., Grebely J., Dore G.J. Hepatitis C virus reinfection and spontaneous clearance of reinfection—the InC3 Study. J Infect Dis. 2015;212:1407–1419.
22. 22. Schulze zur Wiesch J., Ciuffreda D., Lewis-Ximenez L. Broadly directed virus-specific CD4+ T cell responses are primed during acute hepatitis C infection, but rapidly disappear from human blood with viral persistence. J Exp Med. 2012;209:61–75.
23. 23. Rehermann B. Hepatitis C virus versus innate and

adaptive immune responses: a tale of coevolution and coexistence. J Clin Invest. 2009;119:1745–1754.
24. 24. White B., Madden A., Prins M. Assessing the feasibility of hepatitis C virus vaccine trials: results from the Hepatitis C Incidence and Transmission Study-community (HITS-c) vaccine preparedness study. Vaccine. 2014;32:5460–5467.
25. 25. Major M., Gutfraind A., Shekhtman L. Modeling of patient virus titers suggests that availability of a vaccine could reduce hepatitis C virus transmission among injecting drug users. Sci Transl Med. 2018;10:eaao4496.
26. 26. Ward S., Lauer G., Isba R. Cellular immune responses against hepatitis C virus: the evidence base 2002. ClinExpImmunol. 2002;128:195–203.
27. 27. von Delft A., Donnison T.A., Lourenco J. The generation of a simian adenoviral vectored HCV vaccine encoding genetically conserved gene segments to target multiple HCV genotypes. Vaccine. 2018;36:313–321.
28. 28. Youn J.W., Hu Y.W., Tricoche N. Evidence for protection against chronic hepatitis C virus infection in chimpanzees by immunization with replicating recombinant vaccinia virus. J Virol. 2008;82:10896–10905.
29. 29. Elmowalid G.A., Qiao M., Jeong S.H. Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees. ProcNatlAcad Sci U S A. 2007;104:8427–8432.
30. 30. Rollier C., Depla E., Drexhage J.A. Control of heterologous hepatitis C virus infection in chimpanzees is associated with the quality of vaccine-induced peripheral T-helper immune response. J Virol. 2004;78:187–196.
31. 31. Folgori A., Capone S., Ruggeri L. A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees. Nat Med. 2006;12:190–197.
32. 32. Zubkova I., Duan H., Wells F. Hepatitis C virus clearance correlates with HLA-DR expression on proliferating CD8+ T cells in immune-primed chimpanzees. Hepatology. 2014;59:803–813.
33. 33. Houghton M. Prospects for prophylactic and therapeutic vaccines against the hepatitis C viruses. Immunol Rev. 2011;239:99–108.
34. 34. Rollier C.S., Paranhos-Baccala G., Verschoor E.J. Vaccine-induced early control of hepatitis C virus infection in chimpanzees fails to impact on hepatic PD-1 and chronicity. Hepatology. 2007;45:602–613.
35. 35. Colloca S., Barnes E., Folgori A. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. Sci Transl Med. 2012;4:115ra2.
36. 36. Swadling L., Capone S., Antrobus R.D. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. Sci Transl Med. 2014;6:261ra153.
37. 37. Barnes E., Folgori A., Capone S. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Sci Transl Med. 2012;4:115ra1.
38. 38. Osburn W.O., Snider A.E., Wells B.L. Clearance of hepatitis C infection is associated with the early appearance of broad neutralizing antibody responses. Hepatology. 2014;59:2140–2151.
39. 39. Pestka J.M., Zeisel M.B., Blaser E. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. ProcNatlAcad Sci U S A. 2007;104:6025–6030.
40. 40. Logvinoff C., Major M.E., Oldach D. Neutralizing antibody response during acute and chronic hepatitis C virus infection. ProcNatlAcad Sci U S A. 2004;101:10149–10154.
41. 41. Raghuraman S., Park H., Osburn W.O. Spontaneous clearance of chronic hepatitis C virus infection is associated with appearance of neutralizing antibodies and reversal of T-cell exhaustion. J Infect Dis. 2012;205:763–771.

Prof. Muhammad Arif Nadeem
Executive Editor

ORIGINAL ARTICLE

A GROWING MENACE OF MIXED HCV GENOTYPE COINFECTION IN HIGH RISK POPULATION IN PAKISTAN; DIAGNOSTIC AND TREATMENT PERSPECTIVE FOR FUTURE

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Short Title: Increasing HCV Mixed Genotype Infection in Pakistan; A Real Problem for Future

ABSTRACT

Introduction:
Pakistan is a low income country with very limited health care facilities. Although there are guidelines for blood transfusion but due to limited health care infrastructure and funding, these guidelines are not properly followed everywhere. HCV antiviral regimens are fairly genotype dependent and recent data from KPK province of the country showed that mixed HCV genotype infections are increasing in high risk multitransfused individuals.

Objective:
To explore whether increase in mixed HCV genotype infection is limited only in high risk multitransfused individuals or in general population as well.

Method:
1150 HCV positive patients with no history of high risk behavior were enrolled from the KPK province and were checked for HCV infection by PCR and further processed for viral genotyping at Genomic Research Labs and Diagnostics Center, Rawalpindi, Pakistani.

Results:
Among 1150 HCV positive study participants 55.7% were female and 44.3% were male. HCV genotyping results showed that among included patients 1080 (93.9%) were infected with 3a genotype (55.9% female and 44.1% male), 18 (1.6%) were infected with genotype 2a (55.6% female and 44.4% male) and 29 (2.5%) were infected with diagnostically untypable HCV variant (55.1% female and 44.9% male) while only 23 (2%) individuals were infected with mixed HCV genotype (47.8% female and 52.2% male). The results showed that mixed genotypic infections are high risk behavior dependent.

Conclusion:
It is proposed that proper guidelines should be followed for blood transfusions and other risk behaviors to avoid HCV/mixed genotype infection.

Key Words:
HCV; Genotype; Mixed infection; Diagnostic system; Treatment strategies; Pakistan

INTRODUCTION

In Pakistan hepatitis C virus (HCV) burden is very high and almost 11 million individuals are infected with this virus.[1] Pakistan is a low income country having a very low budget for health care system. Although blood screening for blood borne infections is mandatory and recommended for each transfusion but is not followed properly across the country.[2] Because of poor health care setup and very high viral burden in the community, for a multi transfused persons, there is always a chance of getting blood borne infections.[3,4]

HCV due to its error prone nature shows genetic heterogeneity and divided into at least six genotypes and many sub types. Accurate HCV genotyping is very critical for disease management as antiviral therapies are genotype dependent.[5] For single genotype infection most of the current diagnostic methods work well but for mixed genotypic determination one should be very care full and sensitive methods should be used to avoid false negative results. The different methods used for mixed genotype diagnosis are real-time subtype-specific nested reverse transcriptase PCR (nRT-PCR) procedures; heteroduplex mobility assays (HMA); restriction fragment length polymorphism (RFLP) analysis; Sanger sequencing of nRT-PCR amplified products; and next-generation sequencing (NGS).[6] Different diagnostic methods reported to date use 5' untranslated region (UTR), coding region of core protein, highly variable region of envelop glycoprotein (E1-HVR), and a region of viral polymerase (NS5B). The diversity in these targeted regions leads to the

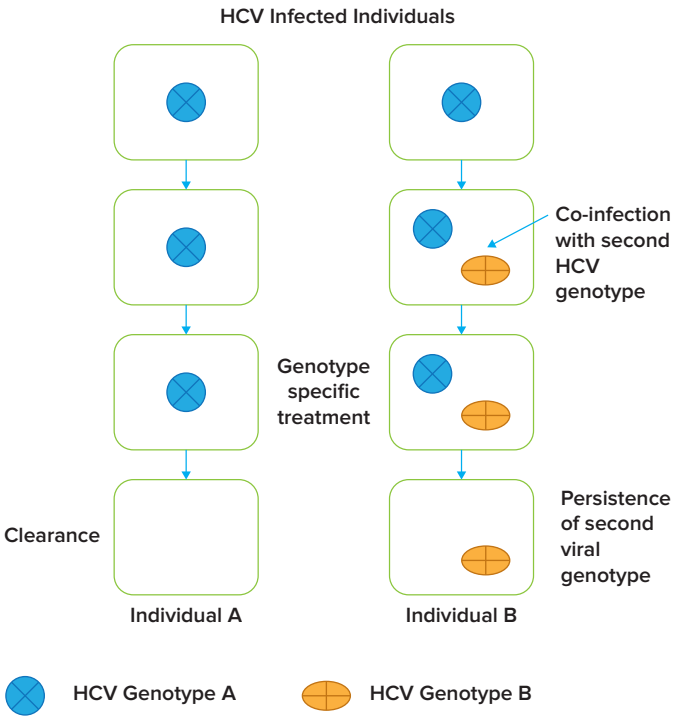

Figure 1: The potential effect of mixed HCV genotypic infection on antiviral therapy. Individual A represents a normal individual while Individual B represents a high-risk individual (multi-transfused person) which is at high risk of second infection. In case single genotype infection, antiviral therapy can clear the sensitive strain. In mixed genotype infection, genotype-specific treatment might only suppress the treatment-sensitive genotype and result in persistence of a treatment-insensitive strain.

identification of different genotypes and subtypes. The choice of method used will help in better identification of mixed genotypes. For example E1-HVR provides enough diversity to differentiate the mix genotypes but NS5B region may not be able to differentiate subtype variations and can leads to amplification of only one viral genotype. In all RT-PCR dependent methods selection of primer choice during reverse transcription and initial round of PCR is very critical and these primers should amplify all targeted viral genotypes. Otherwise one genotype will be amplified and other went un-notice which might results in antiviral therapy resistance/failure.

A recent article by Attaullah and colleagues[7] highlights an important aspect about HCV infection in Pakistan. The study included a large number of multi-transfused individuals and showed a very high number of mixed viral genotype prevalence. From Khyber Pakhtunkhwa (KPK) province, 4607 blood recipient were analyzed and found that among 17.39% anti-HCV positive individuals, 8% were HCV RNA positive. The results of HCV genotype circulation in study cohort are similar to previous finding that genotype 3 is the major prevalent genotype in the country. However this report highlights that there are increasing number of mixed HCV genotypes (7.37%) infection in these high risk individuals as compared with previous reports.[reviewed in 1-4] Detection of multiple HCV variants at a single time point, which are genetically different from each other is called mixed HCV infection. Due to lack of protective immunity during HCV primary infection and ongoing multiple blood transfusions there is a higher chance of superinfection which might results in mixed genotypic infection **(figure 1)**. Current study was aimed to explore whether increase in mixed HCV genotype infection is limited only in high risk multitransfused individuals or in general population as well.

METHODS

HCV positive individuals with no history of multitransfusion, injection drug usage and any other high risk behavior for HCV infection were included in the study. Individuals with chronic HCV infection were included for the study while patients with acute viral infection were excluded. Patients from Khyber Pakhtunkhwa (KPK), Pakistan were checked for HCV infection by PCR and among HCV positive individuals, 1150 were further processed for viral genotyping at Genomic Research Labs and Diagnostics Center, Rawalpindi, Pakistani. This study was approved by the Institutional Bioethics Review Committee (IBRC) of Department of Life sciences, University of Management and Technology, Lahore, Pakistan. Informed written consent was taken from each participant to participate in the study. For infants, informed consent to participate in the study was obtained from their parents or legal guardians prior to enrolling the infants in the study. Serum collection of patients was done from blood and then RNA was extracted according to the manufacturer's protocol by using QIA amp Viral RNA MINI kit (Qiagen-Germany). Amplified DNA products were run on 2 % agarose gel electrophoresis and visualized by using a gel documentation system. All HCV positive samples were then subjected to genotyping using the method of Ohno et al.[8] The amplified PCR product was then electrophoresed on 2 % agarose gel with DNA marker of 100 bp and visualized using gel documentation system. HCV genotype was confirmed on the basis of specific PCR band size.

To compare the results of the study with previous findings from the country, HCV genotyping from last eight years (2010-17) were collected by using different key words. Different sources like PubMed, Google Scholar, PakMediNet (Pakistani Medical Journals and Drugs Database) were used to retrieve the available data.

RESULTS

Among 1150 HCV positive study participants 55.7% were female and 44.3% were male. HCV genotyping results of the current study showed that among included patients 1080 (93.9%) were infected with 3a genotype (55.9% female and 44.1% male), 18 (1.6%) were infected with genotype 2a (55.6% female and 44.4% male) and 29 (2.5%) were infected with diagnostically untypable HCV variant (55.1% female and 44.9% male) while only 23 (2%) individuals were infected with mixed HCV

Table 1: Overall and gender wise HCV genotypic distribution in Khyber Pakhtunkhwa, Pakistan (N=1150)

Genotype	Overall Prevalence		Gender Wise Genotype Distribution			
			Male		Female	
	N	%	N	%	N	%
3A	1080	93.9	476	44.1	604	55.9
2A	18	1.6	8	44.4	10	55.6
Mixed Genotype	23	2	12	52.2	11	47.8
Untypable	29	2.5	13	44.9	16	55.1

genotype (47.8% female and 52.2% male) **(table 1)**. The data showed that prevalence of mixed HCV genotypic infection is comparatively higher in male while all other viral genotypes are more prealent in female.

The previously available data (2010-17) showed that there are 28 studies on prevalence of HCV genotyping in Pakistan. Studies showing the HCV genotyping prevalence in high risk populations were excluded from this analysis. The data examination showed that HCV genotype 3 is the most prevalent viral type in the country followed by diagnostically untypable variants. The mixed viral genotypic infection was observed in 2.25% individuals **(figure 2)**.

DISCUSSION

Hepatitis C Virus antiviral treatment is viral genotype dependent so genotyping is very critical in disease management. In Pakistan HCV genotype 3a is dominantly prevalent and fortunately showed good SVR for conventional interferon based antiviral regimens. The current study also supported that Pakistani HCV patients are predominantly infected with HCV genotype 3a (93.9%) as described by many previous reports.[1-2, 4, 7, 9-15] The prevalence of mixed genotype coinfection in this study is comparable with previous reports from different geographical locations of Pakistan. A comprehensive review of HCV genotype prevalence in Pakistan (2010-2017) showed that 2.25 percent HCV positive individuals have mixed HCV genotypic infection **(figure 2)**. These findings suggested that mixed genotype infection was comparatively higher in high risk multi-transfused individuals might be due to poor health care facilities at the transfusion centers.

There are many reports from Pakistan showing the presence of a large number of diagnostically untypable HCV variants[1-2, 4, 7, 9-17] as found in this study as well. The presence of these diagnostically untypable variants highlights the need of improvements in current methods of HCV genotyping in the country. Accurate genotyping is crucial for designing/planning the treatment strategy of the infected individuals. If the current diagnostic methods are unable to identify the main viral genotypes, there is an ample possibility that these diagnostic procedures might not be able to identify the mixed genotype infections.

HCV antiviral regimens are fairly genotype dependent. From last two decades the major antiviral treatment option against HCV is interferon based. Interferon (plus ribavirin) therapy is genotype dependent and showed different sustained virological response (SVR) against different viral genotypes ranging from 40–50% SVR for genotype 1 and 70–80% in genotype 2 and 3 infections.[18] A recent report showed that peg-interferon plus ribavirin showed SVR rate of 56% for genotype 1, 84% for genotype 2. But for mixed infection (genotype 1+2) 74% patients showed SVR.[19] In mixed genotype infection patients not only SVR rate was lower but also a higher proportion of liver cirrhosis (22%) was observed as compared with single infection patients. These finding showed that the mixed genotype infection should be carefully diagnosed and monitored during treatment. Due to side effects and intolerance of interferon therapy, recently more tolerable and efficient direct acting antiviral (DAA) are approved by FDA. These DAA includes boceprevir and telaprevir (NS3/4A protease inhibitor) (approved in 2011), sofosbuvir (NS5B polymerase inhibitor) (approved in December 2013), ledipasvir (NS5A inhibitor) (approved in October 2015), Grazoprevir (NS3/4A protease inhibitor) and elbasvir (NS5A inhibitor) (approved in January 2016). These DAA regimens have higher SVR (more than 80–90%) but still are genotype dependent.[1, 20] A very recent single case report (Feb. 2017) available in literature regarding the treatment of mixed HCV genotype (1a and 2) infection showed the successful treatment of a compensated cirrhotic patient with sofosbuvir, ledipasvir, and ribavirin.[21] Currently, in Pakistan Sofosbuvir is available at highly discounted price and is the major treatment option along with interferon based regimens. As the major prevalent genotype in Pakistan is 3 so both these antivirals showed a very high SVR.

CONCLUSION

HCV genotype 3a is the most prevalent genotype in Pakistan. The interferon based antivirals and Sofosbuvir bases DAA are available at very cheap price and showed good SVR for this viral genotype. But for other genotypes, mixed infections and for diagnostically untypable ones there is no proper treatment option because of unavailability or very high cost of other genotype dependent DAA. However until the availability of pangenotypic treatment options and addressing the issue of emergence of mixed HCV genotype infection, patients with mixed HCV genotype would still benefit from genotype-specific treatment to avoid liver disease

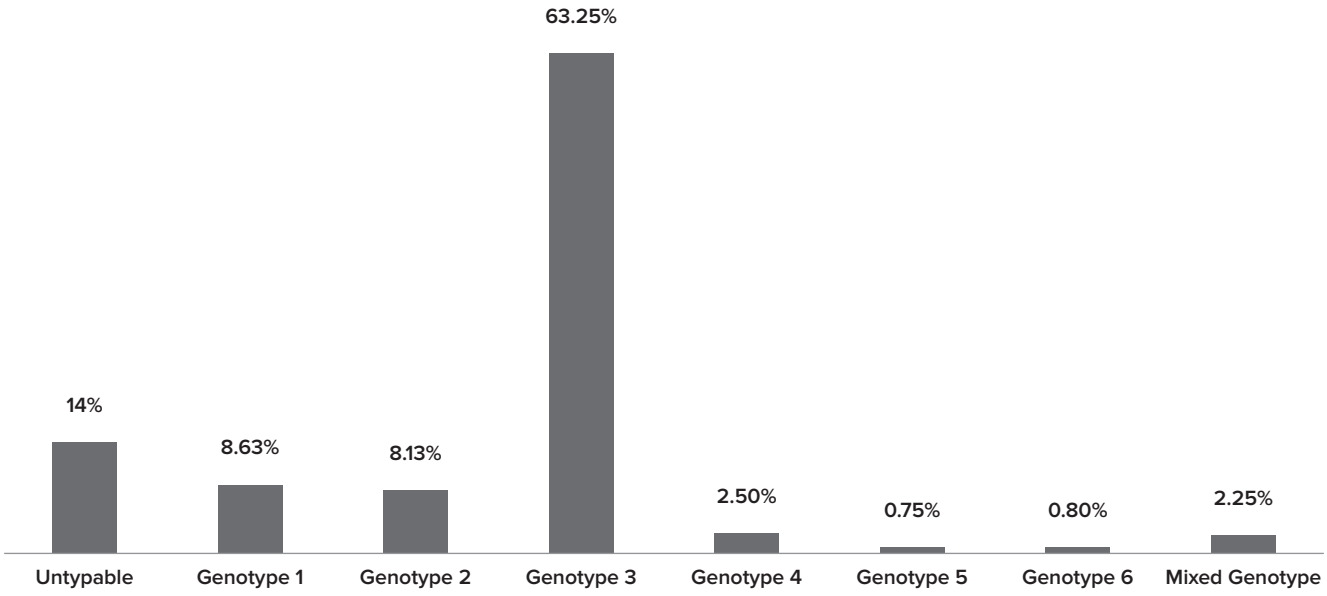

Figure 2: A review of HCV genotype prevalence in Pakistan (2010-2017)

progression.

FUTURE PERSPECTIVES

As Pakistan is low income country with very low health budget and no health insurance system, it is of urgent need to improve the local diagnostic system of the country. Routine genotyping methods if not up to the mark may cause wrong, inaccurate reports due to indeterminate results, mixed infections, wrong subtyping, and even wrong genotyping. These mistakes might be due to nucleotide polymorphisms in HCV that may alter the ability of probe/primer of commercial assays to recognize viral strains. The improvement of local diagnostic system will help to decrease in direct medical costs for the treatment of HCV-infected patients, due to savings for the lower number of retreated patients and lack of disease progression. Future studies regarding the evaluation of diagnostic assays and formulating treatment strategies for HCV mixed genotype infection are highly warranted.

DECLARATIONS SECTION

Ethics approval and consent to participate

The study protocols and informed consent documents were approved by the Institutional Bioethics Review Committee (IBRC) of Department of Life sciences, University of Management and Technology, Lahore. For infants informed consent to participate in the study was obtained from their Parents or legal guardians prior to enrolling the infants in the study.

Consent to publish

Informed written consent for publication was obtained from each participant which stated that the details/images/videos will be freely available on the internet and may be seen by the general public.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Competing interests

The authors declare that they have no competing interests.

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REFERENCES

1. Afzal MS. Hepatitis C Virus and Interferon-Free Antiviral Therapeutics Revolution: Implications for Pakistan. Viral Immunology. 2017;30(4):252-257. doi: 10.1089/vim.2016.0164.
2. Afzal MS, Shah ZH, Ahmed H. Recent HCV genotype changing pattern in the Khyber Pakhtunkhwa province of Pakistan; is it pointing out a forthcoming problem? Braz J Infect Dis. 2016; 20(3):312-3. doi: 10.1016/j.bjid.2015.12.011.
3. Afzal MS, Iqbal MA. Hepatitis C Virus in Pakistan: Community Education Is an Effective Weapon Against the Killer. Viral Immunol. doi: 10.1089/vim.2016.0171.
4. Umer M, Iqbal M. Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data. World J Gastroenterol. 2016 ;22(4):1684-700. doi: 10.3748/wjg.v22.i4.1684.
5. Sperl J, Frankova S, Kreidlova M, Merta D, Tothova M, Spicak J. Combination of sofosbuvir and daclatasvir in the treatment of genotype 3 chronic hepatitis C virus infection in patients on maintenance hemodialysis. Ther Clin Risk Manag. 2017;13:733-738. doi: 10.2147/TCRM.S133983.
6. Cunningham EB, Applegate TL, Lloyd AR, Dore GJ, Grebely J. Mixed HCV infection and reinfection in

people who inject drugs--impact on therapy. Nat Rev GastroenterolHepatol. 2015;12(4):218-30. doi: 10.1038/nrgastro.2015.36.
7. Attaullah S, Khan S, Zahid M. Heterogeneous distribution of HCV genotypes and growing menace of mixed HCV infection in blood recipients in Khyber Pakhtunkhwa. Braz J Infect Dis. 2017. pii: S1413-8670(16)30649-3. doi: 10.1016/j.bjid.2016.12.009.
8. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, Mukaide M, Williams R, Lau JY. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 1997; 35: 201-207
9. Aziz H, Raza A, Murtaza S, Waheed Y, Khalid A, Irfan J, Samra Z, Athar MA. Molecular epidemiology of hepatitis C virus genotypes in different geographical regions of Punjab Province in Pakistan and a phylogenetic analysis. Int J Infect Dis 2013; 17: e247-e253
10. Afridi SQ, Zahid MN, Shabbir MZ, Hussain Z, Mukhtar N, Tipu MY, Akhtar F, Yaqub T. Prevalence of HCV genotypes in district Mardan. Virol J 2013; 10: 90
11. Afridi SQ, Ali MM, Awan F, Zahid MN, Afridi IQ, Afridi SQ, Yaqub T. Molecular epidemiology and viral load of HCV in different regions of Punjab, Pakistan. Virol J 2014; 11: 24
12. Nabi SG, Zaffar G, Sheikh NI, Hassan K, Hassan U. Hepatitis C virus genotypes: a plausible association with viral loads. Indian J Pathol Microbiol 2013; 56: 384-387
13. Afzal MS, Anjum S, Zaidi NU.Changing of HCV clade pattern in Iran; the possible means for something good.Hepat Mon. 2014;14(1):e11879. doi: 10.5812/hepatmon.11879.
14. Afzal MS, Ahmed T, Zaidi NU.Comparison of HCV prevalence in Pakistan and Iran; an insight into future.Hepat Mon. 2014;14(1):e11466. doi: 10.5812/hepatmon.11466.
15. Anjum S, Ali S, Ahmad T, Afzal MS, Waheed Y, Shafi T, Ashraf M, Andleeb S. Sequence and structural analysis of 3' untranslated region of hepatitis C virus, genotype 3a, from Pakistani isolates. Hepat Mon. 2013 May 9;13(5):e8390. doi: 10.5812/hepatmon.8390.
16. Afzal MS, Khan MY, Ammar M, Anjum S, Zaidi NU.Diagnostically untypable hepatitis C virus variants: it is time to resolve the problem.World J Gastroenterol. 2014;20(46):17690-2. doi: 10.3748/wjg.v20.i46.17690.
17. Afzal MS. Predictive potential of IL-28B genetic testing for interferon based hepatitis C virus therapy in Pakistan: Current scenario and future perspective. World J Hepatol. 2016 ;8(26):1116-8. doi: 10.4254/wjh.v8.i26.1116.
18. Raza H, Ahmad T, Afzal MS.HCV, Interferon Therapy Response, Direct Acting Antiviral Therapy Revolution and Pakistan: Future Perspectives.Asian Pac J Cancer Prev. 2015;16(13):5583-4.
19. Lin CC, Wu CH, Chen HL, Lin IT, Wang SY, Wang TE, Wang HY, Shih SC, Bair MJ. Peginterferon alpha-2b plus ribavirin for chronic hepatitis C virus mixed genotype infection. Ann Hepatol. 2014;13(4):350-5.
20. Götte M, Feld JJ. Direct-acting antiviral agents for hepatitis C: structural and mechanistic insights. Nat Rev Gastroenterol Hepatol. 2016 ;13(6):338-51. doi: 10.1038/nrgastro.2016.60.
21. Ahmed Sakr A, Hanifi JM, Valerie Lin M. Successful Treatment of Mixed Hepatitis C Genotypes in a Cirrhotic Patient With an All-Oral, Interferon-Free Regimen. ACG Case Rep J. 2017;4:e16. doi: 10.14309/crj.2017.16.

ORIGINAL ARTICLE

COMPARISON OF IN-HOSPITAL MORTALITY WITH DIFFERENT AGE GROUPS AND REASONS FOR HOSPITALIZATION AMONG LIVER CIRRHOSIS PATIENTS

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ABSTRACT

Objective:
To compare in-hospital mortality with different age groups and reasons for hospitalization among patients suffering liver cirrhosis hospitalized to tertiary care hospital, Gujranwala, Pakistan.

Method:
This cross-sectionalstudy was conducted in the Department of Medicine Unit 1, GMC Teaching Hospital, Gujranwala from June 2016 to May 2017. The data of all liver cirrhosis patients with age greater than 12 years hospitalized from June 2016 to May 2017 was collected prospectively using a structured proforma. The reason for hospitalization as well as the in-hospital death were noted. Age of the patients was categorized into groups. Statistical analysis was performed using SPSS version 25.

Results:
Out of the total of 1304 patients, 627 (48.1%) were male and 677 (51.9%) were female. The maximum %age of patients who died during hospitalization were who presented with hepatic encephalopathy (68.8%, n=141). Amongst different age groups, death was most prevalent in older adults (23.8%, n=62). There was a statistically significant association of mortality amongst hospitalized cirrhotic patients with age group ($p<0.01$) and reasons for hospitalization ($p<0.01$).

Conclusion:
The specific reasons for hospitalization and age group predicts the mortality among hospitalized cirrhotic patients. Hepatic encephalopathy is the commonest reason for hospitalization amongst patients who died. In-hospital mortality was most prevalent in older adults, and least prevalent in young adults. While considering different reasons of hospitalization in liver cirrhosis patients, death rate was the highest in patients who presented withhepatopulmonary syndromeand was the lowest in patients who presented with hepatic hydrothorax.

Key Words:
Liver cirrhosis, In-hospital mortality, Age groups, Hospitalization reasons, SPSS

INTRODUCTION

Liver cirrhosis is highly prevalent in Pakistan,[1] where HCV infection is its commonest etiology.[2] It the major cause of in-hospital mortality now a days in Pakistan.[3] Its overall prevalence worldwide is 4.5% to 9.5%.[4] The reasons for hospitalization of cirrhotic patients include multiple complications of this disease, e.g. upper gastrointestinal bleed (UGIB),[5] hepatic encephalopathy (HE),[6] spontaneous bacterial peritonitis (SBP),[7] infections other than SBP, hepatocellular carcinoma (HCC),[8] tense ascites,[9] hepatic hydrothorax,[10] renal impairment,[11] and hepatopulmonary syndrome (HPS).[12] The percentage of liver cirrhosis patients who died during hospitalization define in-hospital mortality rate.In-hospital mortality among patients suffering liver cirrhosis is high world-wide, ranging from 13.5% to 35%.[13,14] Internationally, the age of the patients can be categorized into five groups. These include childhood if < 13 years, adolescence if 13-18 years, young adults if 19-44years, middle aged adults if 45-65 years, and older adults if >65 years.[15,16] Multiple factors affecting mortality of cirrhotic patients are known, including worse CTP score, worse MELD score, and advanced age.However, till now, correlation of mortality with reasons of hospitalization in our institutes is poorly understood. Therefore, the aim of the present study was to compare in-hospital mortality with different age groups and reasons for hospitalization among patients suffering liver cirrhosis hospitalized to tertiary care hospital, Gujranwala, Pakistan.

METHODOLOGY

This cross-sectional study[17] was conducted in the Department of Medicine Unit 1, GMC Teaching Hospital, Gujranwala from June 2016 to May 2017. Sample size calculation was performed using online Rao soft calculator. With a population size of 20000, response distribution of 50% and confidence interval of 95%, the minimum recommended sample size was 377. The written

informed consent was taken from all patients. The data was collected prospectively by purposive sampling using a structured proforma. All the diagnosed CLD patients with age greater than 12 years who were hospitalized for different complications of liver cirrhosis were included in this study.The outcome of the hospitalization of all the patients in term of recovery or death was also noted. For the correlation of some factors affecting this outcome of hospitalization, the age of the patients was categorized into childhood if < 13 years, adolescence if 13-18 years, young adults if 19-44years, middle aged adults if 45-65 years, and older adults if >65 years.[15,16] Statistical analysis was performed using the Statistical Package for Social Science (SPSS), version 25. Age of the patients was the only quantitative variable, while gender, age groups, and reason for hospitalization were the qualitative variables. During descriptive interpretation of data, continuous variables were expressed as mean and standard deviation. Frequencies and percentages were computed for different categorical variables. The chi-square test or Fisher's exact test were used for data analysis. All p-values were two sided and considered as statistically significant if < 0.05. Odds ratios and confidence interval for predictors of mortality were calculated.

RESULTS

Amongst 1304 hospitalized liver cirrhosis patients, 627 (48.1%) were male and 677 (51.9%) were female. Amongst different age groups, mortality was most prevalent in older adults, and least prevalent in young adults. 23.8% (62 out of 261) older adults, 15.3% (122 out of 797) middle aged adults, 13.3% (2 out of 15) adolescents, and 8.2% (19 out of 231) young adults died, while remaining patients got discharge, referred or leaved against medical advice. This association of death rate with different age groups was statistically significant (p<0.01). Amongst the different reasons of hospitalization in liver cirrhosis patients, death rate was the highest in patients who presented with HPS and was the lowest in patients who presented with hepatic hydrothorax. Those CLD patients were hospitalized for HPS, HE, RI, UGIB, TA, HCC, SBPI, and HH, and the death prevalence among them was 58.3% (7 out of 12), 48% (141 out of 294), 13% (7 out of 54), 7.3% (37out of 509), 5.6% (5 out of 89), 3% (2 out of 66), 2.3% (6 out of 256), and 0% (0 out of 23) respectively. The association of reasons for hospitalization with mortality was statistically significant (p<0.01) **(table 1)**. The maximum % age of patients who died during hospitalization were who presented with HE. Amongst the patients who died 68.8% (n=141) had HE, 18% (n=37) had UGIB, 3.4% (n=7) had HPS, 3.4% (n=7) had RI, 2.9% (n=6) had SBPI, 2.4% (n=5) had TA, and 1% (n=2) had HCC **(figure 1)**.

DISCUSSION

In 2016, Zubieta-Rodriguez and colleagues[18] from Colombia found that the most frequent cause of death in admitted cirrhotic patients was septic shock (68.4%) followed by bleeding/ hypovolemic shock (10/5%). In our study, patients who died during hospitalization, 68.8% had HE, 18% had UGIB, and remaining 13.2% had HPS, RI, TA etc. Hence, we can say that that loss of consciousness because of HE, sepsis or volume loss is always the chief reason of death world-wide among hospitalized cirrhotic patients.

Zubieta-Rodriguez et al observed a mortality rate of 23.5%among hospitalized cirrhotic patients. In 2009, Muhammad A Alsultan and colleagues[14] from Riyadh, Saudi Arabia reported 35% inpatient mortalityamong cirrhotic patients. In 2011, Cristal L. Brown and his colleagues[13] from North Carolina, USA demonstrated 13.5%mortality

Table 1: Comparison of in-hospital mortality with age groups and reasons for hospitalization among liver cirrhosis patients. (n = 1304)

Factors	In Hospital Mortality		Total	p-value
	Yes	No		
Age Groups:				
Adolescence	2 (13.3%)	13 (86.7%)	15	<0.01
Young adults	19 (8.2%)	212 (91.8%)	231	
Middle aged adults	122 (15.3%)	675 (84.7%)	797	
Older adults	62 (23.8%)	199 (76.2%)	26	
Reason for Hospitalization:				
Hepatic encephalopathy	141 (48%)	153 (52%)	294	<0.01
UGIB	37 (7.3%)	472 (92.7%)	509	
HPS	7 (58.3%)	5 (41.7%)	12	
Renal impairment	7 (13%)	47 (87%)	54	
SPB & other infections	6 (2.3%)	251 (97.7%)	257	
Tense ascites	5 (5.6%)	84 (94.4%)	89	
HCC	2 (3%)	64 (97%)	66	
Hepatic hydrothorax	0 (0%)	23 (100%)	23	

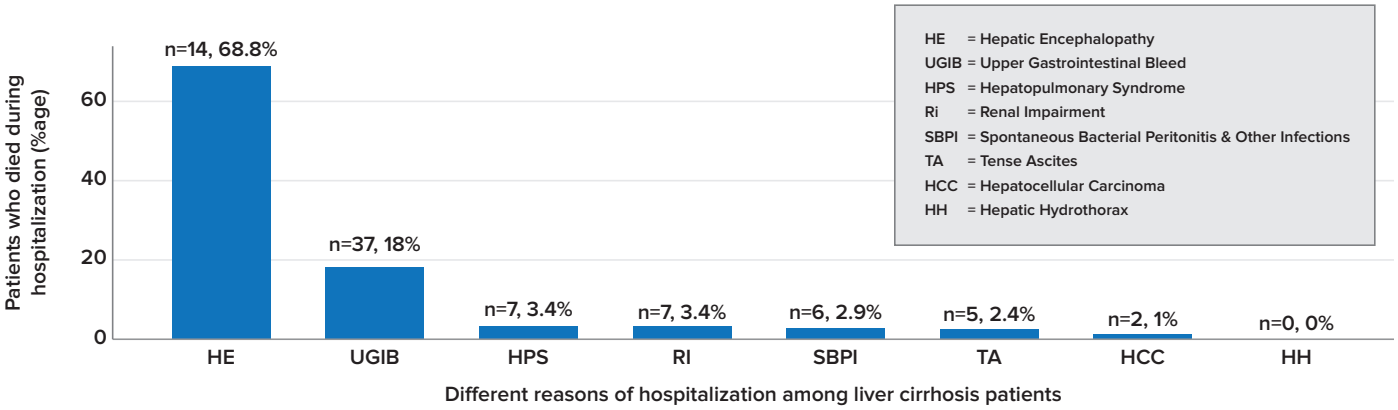

Figure 1: Different reasons of hospitalization among patients who died of liver cirrhosis (n=105/1304)

rate among hospitalized cirrhotic patients. In our study, we found 15.72% (n=205/1304) mortality rate among hospitalized cirrhotic patients. Hence, liver cirrhosis has a high inpatient mortality rate world-wide. Multiple factors affect the outcome of hospitalization in these admitted cirrhotic patients. Muhammad A Alsultan et all observed worse outcome of hospitalization in cirrhotic patients who had worse CTP score, worse MELD score, and advanced age.[14] They also found that advanced age (p=0.004) was an independent risk factor for the mortality of cirrhotic patients. Similarly, Cheng-Yi Chenand colleagues[19] found that age >75 years was significantly correlated with in-hospital mortality. In our study, we correlated in-hospital mortality with different age groups. Mortality was 13.3%, 8.2%, 15.3%, and 23.8% in adolescents, young adults, middle aged adults, and older adults respectively. Hence, in our population, death was most prevalent in older adults with age > 65 years suffering liver cirrhosis. It seems that advanced age is always a risk factor for the mortality of cirrhotic patients world-wide. In addition to advanced age, our study also demonstrated that specific reason for hospitalization in the patients were also associated with higher mortality among cirrhotic patients. The cirrhotic patients who were hospitalized for HPS, HE, RI, UGIB, TA, HCC, and SBP had a mortalityrate of 58.3%, 48%, 13%, 7.3%, 5.6%, 3%, and 2.3% respectively.Gokhan Tumgor also mentioned very poor prognosis with hepatopulmonary syndrome among liver cirrhosis patients without liver transplantation.[20] Hence, advanced age, and specific reason for hospitalization like hepatopulmonary syndrome predict the mortality among hospitalized cirrhotic patients.

CONCLUSION

The specific reasons for hospitalization and age group predicts the mortality among hospitalized cirrhotic patients. Hepatic encephalopathy is the commonest reason for hospitalization amongst patients who died. In-hospital mortality was most prevalent in older adults, and least prevalent in young adults. While considering different reasons of hospitalization in liver cirrhosis patients, death rate was the highest in patients who presented withhepatopulmonary syndrome and was the lowest in patients who presented with hepatic hydrothorax.

REFERENCES

1. Shah S, Mashia S, Younus M, Ghauri A, Ejaz R, Alshalabi H, et al. Hepatic Cirrhosis - Disease Burden. Journal of Rawalpindi Medical College Students Supplement 2015; 19(1):1 7-20.
2. Fahim-Ullah, Khan S, Afridi A, and Rahman S. Frequency of different causes of cirrhosis liver in local population. Gomal Journal of Medical Sciences 2012;10 (2):178–181.
3. Khokhar N, Niazi SA.Chronic liver disease related mortality pattern in Northern Pakistan. J Coll Physicians Surg Pak. 2003 Sep;13(9):495-7.
4. Melato M, Sasso F, Zanconati F. Liver cirrhosis and liver cancer. A study of their relationship in 2563 autopsies. Zentralbl Pathol 1993; 139: 25–30.
5. Saltzman J. Acute Upper Gastrointestinal Bleeding. In: Greenberger N, Blumberg R, Burakoff R, editors. Current diagnosis and treatment Gastroenterology, Hepatology, & Endoscopy. 3rd Ed. New York: Mc Graw-Hill; 2016: 375-6
6. Ferenci P, Lockwood A, Mullen K, et al: Hepatic encephalopathy-definition, nomenclature, diagnosis, and quantification: Final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology 2002; 35:716-21.
7. Hoefs JC, Canawati HN, Sapico FL, Hopkins RR, Weiner J, Montgomerie JZ. Spontaneous bacterial peritonitis. Hepatology 1982;2: 399-407.
8. Ioannou G, Splan M, Weiss N, et al: Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol 2007; 5:938-45.
9. Peltekian KM, Wong F, Liu PP, Logan AG, Sherman M, Blendis LM. Cardiovascular, renal and neurohumoral responses to single large-volume paracentesis in cirrhotic patients with diuretic-resistant ascites. Am J Gastroenterol 1997; 92: 394-399.
10. Strauss RM, Boyer TD. Hepatic hydrothorax. Sem Liv Dis 1997;17: 227-232
11. Salerno F, Gerbes A, Gines P, Wong F, Arroyo V. Diagnosis, prevention and treatment of the

hepatorenal syndrome in cirrhosis: a consensus workshop of the international ascites club. Gut 2007;56: 1310-1318.
12. Rodriguez-Roisin R, Krowka MJ, Herve P, et al: ERS Task Force Pulmonary-Hepatic Vascular Disorders Scientific Committee. Eur Respir J 2004; 24:861-80.
13. Brown CL, Hammill BG, Qualls LG, Curtis LH, and Muir AJ. Significant Morbidity and Mortality Among Hospitalized End-Stage Liver Disease Patients in Medicare. J Pain Symptom Manage 2016; 52(3): 412-419.
14. Alsultan MA, Alrshed RS, Aljumah AA, Baharoon SA, Arabi YM, et al. In-Hospital Mortality Among a Cohort of Cirrhotic Patients Admitted to a Tertiary Hospital. Saudi J Gastroenterol 2011; 17(6): 387-390.
15. https://en.oxforddictionaries.com/definition/us/middle_age
16. www.widener.edu/about/campus_resources/wolfgram.../life_span_chart_final.pdf
17. Tasneem AA and Luck NH. Acute-On-Chronic Liver Failure: Causes, Clinical Characteristics and Predictors of Mortality. J Coll Physicians Surg Pak. 2017 Jan;27(1):8-12.
18. Zubieta-Rodríguez R, Gómez-Correa J, Rodríguez-Amaya R, Ariza-Mejia KA and Toloza-Cuta NA. Hospital mortality in cirrhotic patients at a tertiary care center. Revista de Gastroenterología de México. 2017; 82(3): 203 – 209.
19. Chen CY, Wu CJ, Pan CF, Chen HH, and Chen YW. Influence of age on critically ill patients with cirrhosis. International Journal of Gerontology 2015; 9(4): 233-238.
20. Tumgor G. Cirrhosis and hepatopulmonary syndrome. World J Gastroenterol. 2014;20(10):2586-94.

ORIGINAL ARTICLE

EPITOPE MAPPING OF HCV GLYCOPROTEINS E1 AND E2 FOR POTENTIAL VACCINE DESIGN; AN IN SILICO APPROACH

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Running Title: HCV Vaccine; An Insilico Approach

ABSTRACT

Introduction:
Due to nature of being fallible and error prone, HCV RNA escape of immune system and replicate more to cause chronic hepatitis. The development of HCV vaccine is a need of hour for the successful eradication of infection.

Objective:
The main purpose of this study was to make a pan-genotypic vaccine by predicting the epitopes of HCV glycoproteins (E1, E2) which will act as immunogens.

Method:
The study analyzed glycoproteins as potential vaccine candidates. Available glycoproteins sequences of different viral genotypes were aligned to develop consensus sequence of the the proposed vaccine. Epitopes sequences were predicted by IEDB software, analyzed through Vaxijen server for potential antigenicity and then I-Tasser software was used to visualize the predicted epitopes.

Results:
Total 52 E1 and E2 protein sequences were used to make a direct comparison and to generate a consensus sequence of both. 23 antigenic epitopes for HCV E1 and 29 antigenic epitopes for HCV E2 have been seen as immunogenic. These were found to be more potential vaccine candidates as compared to other derived epitopes because of their surface presence in hydrophilic region rather than in deep pocket.

Conclusion:
It is important to develop effective vaccines which target the multiple antigenic components of the virus. These identified immunogenic epitopes can be useful in the development of a pan-genotypic vaccine against HCV.

Key Words:
Epitope; Glycoproteins; HCV; Immunity; Insilico; Vaccine

INTRODUCTION

Hepatitis C virus (HCV) is a prime cause of hepatitis related morbidities and mortalities.[1] It has been reported by World Health Organization (WHO) that Europe and Eastern Mediterranean regions are utmost affected by the HCV infection, prevalence rate ranges 1.5% to 2.3% respectively. While other regions of world varies in HCV prevalence from 0.5% to 1%.[2] Recent studies have shown that over the last 15 years HCV seroprevalence burden has been increased (2.8%) which is nearly equal to 185 million infections globally.[3]

Africa is falling under the high rated region of HCV incidence with 2.9% prevalence rate.[4] In contrast to this, North America and Western Europe comes under low prevalence rated regions. Developed countries have reported comparatively low seroprevalence rate of HCV, it includes Germany 0.6%, Canada 0.8%, France and Australia 1.1%.[5-9]

The highest seroprevalence rate of 22% has reported in Egypt.[10] Pakistan is a low income and developing country with quite high HCV seroprevalence rate ranging 2.4% to 6.5%.[11-14]

HCV is a spherical shaped single stranded RNA virus belongs to Flaviviridae family and Hepacivirus genus.[15] Due to its nature of being fallible it has the ability to escape immune system surveillance. This escape of immune system helps the virus to replicate more and to be a chief causative agent of chronic hepatitis. Cytotoxic (CD8+) and helper T (CD4+) lymphocytes play their role in abolition of virus. These T cell subsets are associated with the clearance of virus infection. CD4+ and CD8+ lymphocytes can enhance the clearance outcome effect by their functional ability. The progression of chronic infection directing to fibrosis and inflammation is mainly stimulated by many genetic and environmental factors. Key risk factors for HCV incidence are unsafe medical and dental procedures, multiple sex partners, unsafe sex, unsafe syringes usage, reuse of blades in barber shops,

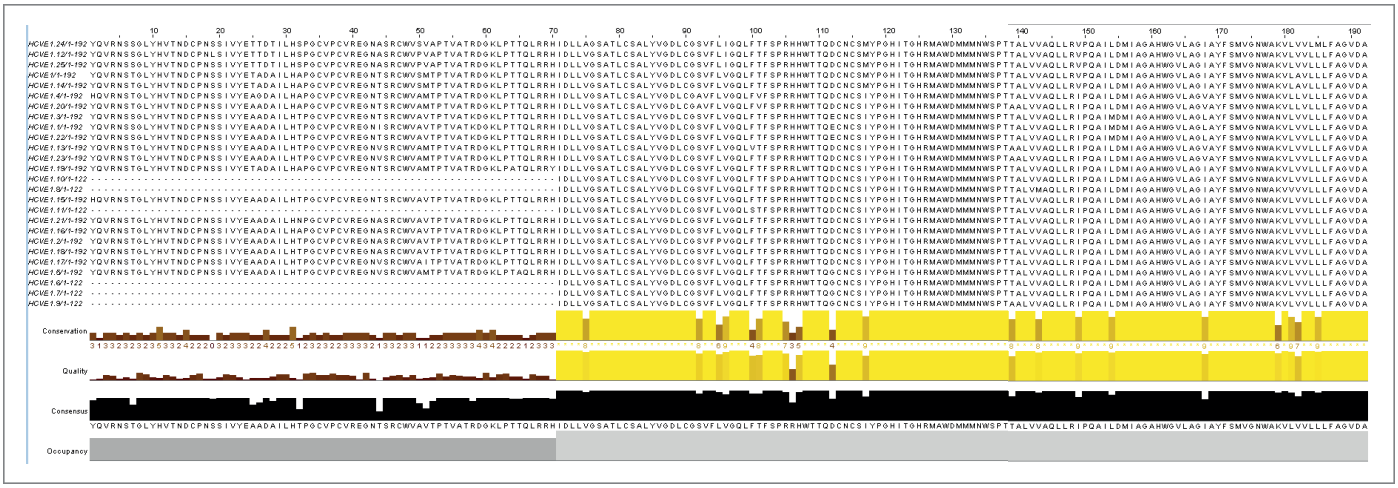

Figure 1: Consensus sequence of HCV envelop protein 1 (E1) made by Jalview software

and blood transfusion[16]

It is a great mystery to successfully control HCV infection. The cell culture and in vivo studies showed that CD4+ and CD8+ T cells play a major role in HCV clearance by producing long lasting and vigorous reaction responses.[17-18] Currently there is no vaccine available for HCV prevention. There is a need of an effective and operative vaccine to eliminate HCV successfully. But due to the error prone nature of the virus and incomplete natural immunity, it is a notable challenge for the researchers to develop an effective vaccine against HCV. Due to the large number of variations in structural and genetic makeup of the virus, it is getting really hard to develop a vaccine against HCV.

It is unsafe to use a whole HCV live attenuated or inactivated vaccines due to the chances of the virus relapse. So it is a need of an hour to develop an efficient vaccine. An ultimate goal of a vaccine is to prevent HCV infection effectively by providing neutralizing antibody responses[19-20]

There are different HCV genotypes and all have differential pathogenesis. Recent antiviral regimens are genotypic specific which make them specific to use against a specific genotype. This study has been designed while keeping all these hurdles under consideration. The main purpose of this study was to make a pan-genotypic vaccine by predicting the epitopes of HCV glycoproteins (E1, E2) which will act as immunogens.

MATERIALS AND METHODS

Sequence Retrieval

HCV glycoprotein (E1, E2) sequences were retrieved from the National Center for Biotechnology Information (NCBI) and UniProt. The data base was searched to get the maximum similar and comparable protein sequences from the database. Multiple sequence alignment was created by using CLUSTAL OMEGA (https://www.ebi.ac.uk/Tools/msa/clustalo/) with keeping settings default.

Consensus sequence was produced by using JALVIEW software and aligned sequences were also visualized **(figure 1 and figure 2)**.

Prediction of T-Cell Binding epitopes

HLA-I and HLA-II binding is required for T cell epitope prediction. Propred I (http://crdd.osdd.net/raghava/propred1/) was used to predict HLA-I binding epitopes in consensus sequences of E1 and E2. Threshold value of 4% was selected and 5% default threshold value was selected for proteasome and immune-proteosome filters to get the most out of binding epitopes efficiency. Epitopes which can bind to 47 HLA- I alleles were determined by Propred-I. Propred (http://crdd.osdd.net/raghava/propred/) was used to determine HLA-II binding epitopes with default settings and has a property to predict epitopes for 51 HLA-II alleles.

Antigenicity Analysis

Vaxijen server version 2.0 was used to determine the antigenicity score of predicted epitopes for analysis. Threshold value of 0.01 was given to eliminate the non-antigenic sequences. This online tool avoids the rearrangement of amino acids which helps to perform the prediction of protective antigens.

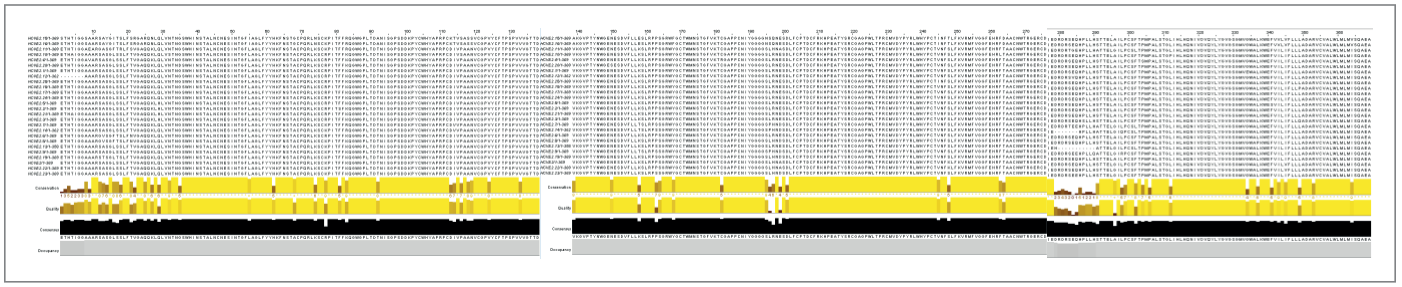

Figure 2: Consensus sequence of HCV envelop protein 2 (E2) made by Jalview software

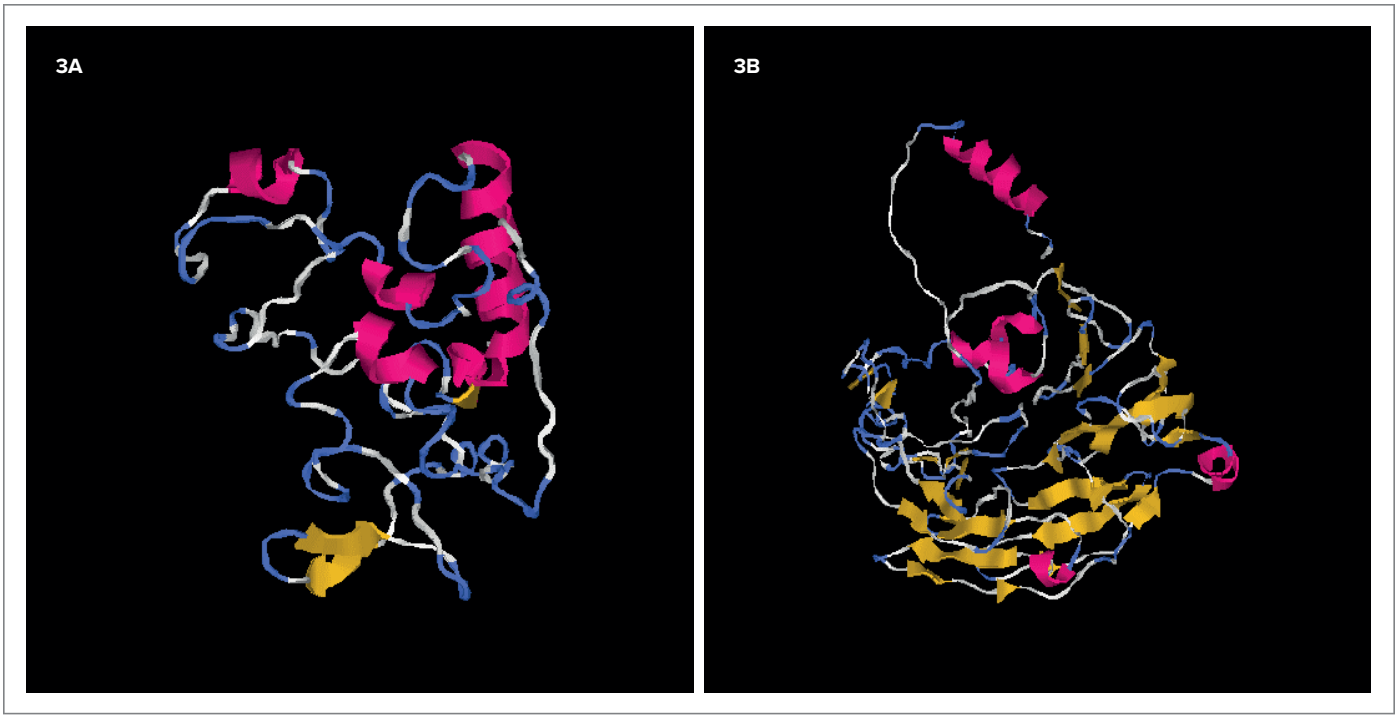

Figure 3: (3A) 3-dimensional model of gpE1 (3B) 3-dimensional model of gpE2

Immunogenicity Prediction of MHC-I

Consensus sequence of HCV glycoproteins (E1, E2) were used in MHC-I binding of Immune Epitope Database (IEDB) for the analysis of epitopes and more peptides prediction. Thousands of peptides were formed which were then scrutinized on the basis of percentile ranking and inhibition concentration (IC-50) value.

Structure Visualization

Potential antigenic and immunogenic peptide candidates were visualized structurally on Iterative Threading Assembly Refinement (I-TASSER) (https://zhanglab.ccmb.med.umich.edu/I-TASSER/). Three dimensional structures of protein molecules can be predicted by this software **(figure 3)**. Structure templates were detected from the Protein Data Bank (PDB) by a technique named as Threading or Fold Recognition.

RESULTS

By using different data bases, total 52 E1 and E2 protein sequences were used to make a direct comparison and to generate a consensus sequence of both. 23 antigenic epitopes for HCV E1 **(table 1)** out of 1000 peptides have been predicted as immunogenic from the consensus sequence. While 29 antigenic epitopes for HCV E2 **(table 2)** out of 1000 peptides have been predicted. All the predicted epitopes were from the conserved regions. Two epitopes MMNWSPTTA, MMMNWSPTT for HCV E1 protein and two epitopes HTIGGAAAR, MFVGGFEHR for HCV E2 protein were further selected as a potential vaccine candidate on the basis of high antigenicity score.

The epitopes for HCV E-1, MMNWSPTTA at position 132 - 140 amino acid **(figure 4A)** with antigenicity score of 1.9709 and MMMNWSPTT at position 131- 139 amino acid **(figure 4B)** with antigenicity score of 1.5722 were seen immunogenic. While at the other side epitopes for HCV E-2, MFVGGFEHR at position 254 – 262 amino acid **(figure 4C)** with antigenicity score of 1.4817 while epitope HTIGGAAAR at position 3 - 11 amino acid **(figure 4D)** with antigenicity score 1.4786 were seen immunogenic.

I-Tasser provided the proposed 3D structures of the HCV glycoprotein (E1, E2) and the predicted epitopes with the best antigenicity cut off value were visualized. These four predicted peptide sequences of E1 and E2 proteins were found immunogenic on the basis of their high antigenicity score and good binding affinity with HLAs. These were found to be more potential vaccine candidates as compared to other derived epitopes because of their surface presence in hydrophilic region rather than in deep pocket. These four peptides were more prone to degradation on the basis of their availability on surface, hence showed more immunogenicity.

DISCUSSION

HCV is having an increased burden of infections which is nearly equal to 185 million globally.[3] Highest prevalence rated countries are located in Africa and Asia. It has been seen that HCV infection rate is quite higher in under developing countries. Pakistan is one of the under developing countries and having a very high rate of HCV seroprevalence.[11-16]

Due to the high variation in the genome and error prone nature makes very difficult to develop a vaccine against HCV virus.[21-22] Antigenic epitopes have found out by insilico prediction of T cell epitopes which can then be incorporated to formulate a vaccine. A number of online available software tools are present to find the HLA binding epitopes. Chronic infection of HCV leads to liver fibrosis and cancer; can be prevented by HCV vaccines.

Table 1: MHC-I binding of predicted T-cell epitope sequences of HCV E1 protein with antigenicity score

Sr.	Allele	Position	Length	Epitope Sequence	Antigenicity Score
1	HLA-B*53:01	127-135	9	MAWDMMMNW	0.7088
2	HLA-A*02:03	131-140	10	MMMNWSPTTA	1.4469
3	HLA-B*58:01	126-135	10	RMAWDMMMNW	0.8194
4	HLA-B*57:01	127-135	9	MAWDMMMNW	0.7088
5	HLA-A*02:01	131-140	10	MMMNWSPTTA	1.4469
6	HLA-A*02:06	156-164	9	MIAGAHWGV	0.4273
7	HLA-A*02:06	181-190	10	LVVLLLFAGV	0.3291
8	HLA-A*68:02	43-51	9	NTSRCWVAV	0.6696
9	HLA-A*68:02	156-164	9	MIAGAHWGV	0.4273
10	HLA-B*58:01	127-135	9	MAWDMMMNW	0.7088
11	HLA-B*57:01	100-108	9	FTFSPRRHW	0.7246
12	HLA-A*30:02	1 - 10	10	YQVRNSTGLY	0.193
13	HLA-A*32:01	179-187	9	KVLVVLLLF	0.2984
14	HLA-B*58:01	179-187	9	KVLVVLLLF	0.2984
15	HLA-B*35:01	162-170	9	WGVLAGIAY	0.4615
16	HLA-A*02:03	132-140	9	MMNWSPTTA	1.9709
17	HLA-A*31:01	97-105	9	GQLFTFSPR	1.1806
18	HLA-A*68:02	138-146	9	TTALVVAQL	0.7375
19	HLA-A*11:01	171-179	9	FSMVGNWAK	0.1588
20	HLA-A*02:06	182-190	9	VVLLLFAGV	0.2707
21	HLA-A*02:01	131-139	9	MMMNWSPTT	1.5722
22	HLA-A*68:01	31-40	10	HTPGCVPCVR	1.0702
23	HLA-A*68:01	139-148	10	TALVVAQLLR	0.226

Unfortunately there is no vaccine available for HCV unlike hepatitis A virus and hepatitis B virus.[23-24]

In this study, sequences of gpE1 and gpE2 have been taken because these two proteins are very critical regarding virus attachment and cell fusion.[25-26] Therefore glycoprotein is an excellent target for the formulation of a prophylactic vaccine.[27] Previous studies reported 3D structure, epitope analysis and homology modeling of Pakistani isolated HCV E1 protein. They found that E1 epitopes from Pakistani isolates are mostly conserved as compared with HCV variants from other countries.[28-29] Our study focuses on the epitope analysis on the basis of immunogenicity and antigenicity. To get the maximum benefits from the available technology, this is not specified to Pakistani HCV isolates only but providing overall potential vaccine candidates.

Arashkia et al. (2010)[30] reported T cell immune response analysis for gpE2. Insilico predicted immune dominant peptides showed a promising response in mice. This study showed the potential and importance of insilico analysis for potential vaccine development. So the current study included the HCV E1 and E2 proteins from all viral genotypes for their MHC class-I binding ability to get a pan-genotypic vaccine.

All the sequences of HCV glycoproteins E1 and E2 have been analyzed through alignment and insilico approaches. A consensus sequence of query proteins was developed and T cell epitopes were predicted to find the antigenic peptides which were mostly from the conserved region. Epitopes were projected to integrate in a vaccine formulation to meet the assorted and diverse human population. GpE1 epitope MMNWSPTTA at position 132- 140 amino acid was observed to have significant antigenicity and immunogenicity score of 1.9709. While the gpE2 epitope MFVGGFEHR at position 254- 262 amino acid with antigenicity score of 1.4817 was observed to have substantial immunogenicity. These potential vaccine candidates should be considered for further experimental studies. The already available HCV antiviral drugs do not treat all the patients with equal efficiency. So

Table 2: MHC-I binding of predicted T-cell epitope sequences of HCV E2 protein with antigenicity score

Sr.	Allele	Position	Length	Epitope Sequence	Antigenicity Score
1	HLA-B*40:01	339-347	9	WEFVILIFL	0.582
2	HLA-B*40:01	339-348	10	WEFVILIFLL	0.5512
3	HLA-B*40:01	148-156	9	GENESDVFL	0.158
4	HLA-A*02:03	324-333	10	YLYGVGSGMV	0.292
5	HLA-A*68:01	3 - 11	9	HTIGGAAAR	1.4786
6	HLA-A*68:01	153-161	9	DVFLLKSLR	0.8269
7	HLA-A*68:01	263-271	9	FTAACNWTR	0.3385
8	HLA-A*11:01	74-83	10	KSCRPITFFK	0.5777
9	HLA-B*53:01	96-105	10	GPSDDKPYCW	1.1411
10	HLA-B*53:01	241-250	10	YPCTVNFSLF	0.221
11	HLA-B*40:01	148-157	10	GENESDVFLL	0.2058
12	HLA-A*33:01	153-161	9	DVFLLKSLR	0.8269
13	HLA-A*33:01	263-271	9	FTAACNWTR	0.3385
14	HLA-B*07:02	303-312	10	TPMPALSTGL	0.469
15	HLA-A*02:01	237-245	9	RLWHYPCTV	0.41
16	HLA-A*23:01	338-346	9	KWEFVILIF	1.1774
17	HLA-A*31:01	253-262	10	RMFVGGFEHR	1.2164
18	HLA-A*33:01	201-210	10	DLFCPTDCFR	0.1068
19	HLA-A*23:01	238-247	10	LWHYPCTVNF	1.0029
20	HLA-A*33:01	254-262	9	MFVGGFEHR	1.4817
21	HLA-A*02:01	346-354	9	FLLLADARV	0.7825
22	HLA-B*07:02	305-313	9	MPALSTGLI	0.3991
23	HLA-A*03:01	74-83	10	KSCRPITFFK	0.5777
24	HLA-A*31:01	74-83	10	KSCRPITFFK	0.5777
25	HLA-A*31:01	262-271	10	RFTAACNWTR	0.5128
26	HLA-A*11:01	66-74	9	STACPQRLK	0.8745
27	HLA-A*02:03	359-367	9	WLMLMISQA	0.4667
28	HLA-A*68:02	1- 9	9	ETHTIGGAA	0.2947
29	HLA-A*68:02	244-252	9	TVNFSLFKV	0.4347

hereby, prophylactic vaccine development against HCV infection embodies a cost effective and resourceful way to manage the disease incidence.

Much more studies are required to understand the HCV life cycle, disease pathogenesis, immune response against HCV and how HCV evades host immune response. The major limitation of the study is the unavailability of the in vivo and in vitro experimental studies to proof either these antigenic epitopes will induce any immune response. All the epitopes have been predicted by insilico techniques therefore wet lab verification is required to determine the actual effectiveness, immunogenicity and stability of all the peptides. In near future, more data will be available regarding T cell epitopes which will help researchers to develop and focus on the specific sides of the antiviral immunity.

CONCLUSION

It is important to develop effective vaccines which target the multiple antigenic components of the virus. These effective vaccines will help the immune system towards protecting the host from the viral infection. Hence, this

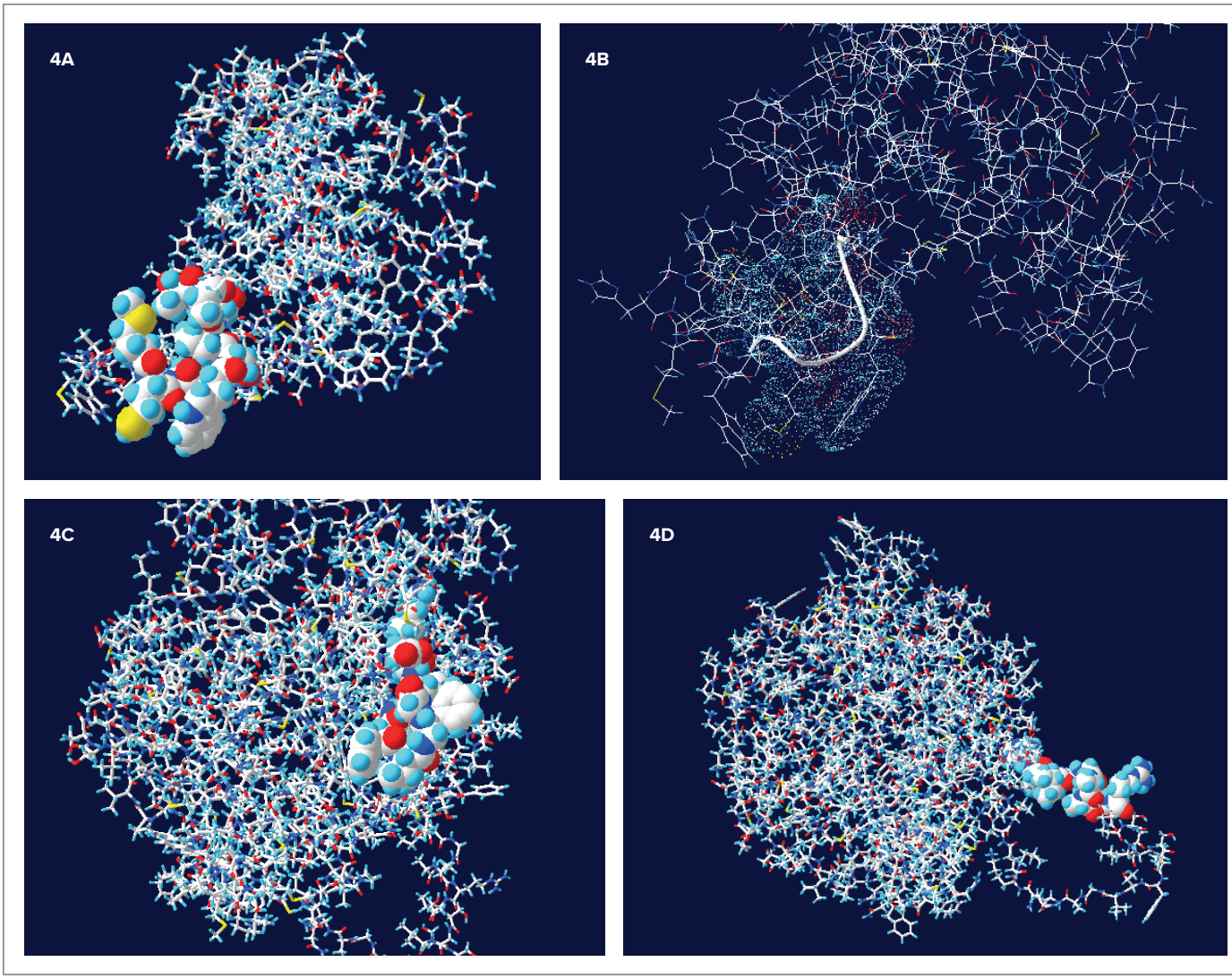

Figure: (4A) GpE1 epitope MMNWSPTTA representing amino acid sequence on the hydrophilic region (4B) GpE1 epitope MMMNWSPTT with amino positions showing in the form of white ribbon (4C) GpE2 epitope MFVGGFEHR present on the outer surface (4D) GpE2 epitope HTIGGAAAR representing amino acid sequence at the outer surface, more prone to degradation

study was conducted to determine the antigenic epitopes of HCV glycoproteins (E1 and E2). Results exposed the potential antigenic Tc-cell epitopes which can raise the desired immune response against HCV. In order to protect from this endemic threat, these epitopes can be useful in the development of a pan-genotypic vaccine against HCV.

REFERENCES

1. Cooke, G. S., Lemoine, M., Thursz, M., Gore, C., Swan, T., Kamarulzaman, A., & Ford, N. (2013). Viral hepatitis and the Global Burden of Disease: a need to regroup. Journal of viral hepatitis, 20(9), 600-601.
2. World Health Organization. http://www.who.int/mediacentre/factsheets/fs164/en/ Accessed on 07-01-2018
3. Mohd Hanafiah, K., Groeger, J., Flaxman, A. D., & Wiersma, S. T. (2013). Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology, 57(4), 1333-1342.
4. Petruzziello, A., Marigliano, S., Loquercio, G., Cozzolino, A., & Cacciapuoti, C. (2016). Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. World journal of gastroenterology, 22(34), 7824.
5. Palitzsch, K. D., Hottenträger, B., Schlottmann, K., Frick, E., Holstege, A., Schölmerich, J., & Jilg, W. (1999). Prevalence of antibodies against hepatitis C virus in the adult German population. European journal of gastroenterology & hepatology, 11(11), 1215-1220.
6. Zou, S., Tepper, M., & El Saadany, S. (2000). Prediction of hepatitis C burden in Canada. Canadian Journal of Gastroenterology and Hepatology, 14(7), 575-580.
7. Desenclos JC. Epidemiology of hepatitis C. Revue du Praticien 2000; 50: 1066–70.
8. Law, M. G., Dore, G. J., Bath, N., Thompson, S., Crofts, N., Dolan, K., ... & Powell, E. (2003). Modelling

hepatitis C virus incidence, prevalence and long-term sequelae in Australia, 2001. International Journal of Epidemiology, 32(5), 717-724.
9. Australian census, 2001, total population. http://www.abs.gov.au/ausstats/abs%40census.nsf/ddc9b4f92657325cca256c3e000bdbaf/7dd97c937216e32fca256bbe008371f0!OpenDocument#Census Counts (accessed July 25, 2005).
10. Frank, C., Mohamed, M. K., Strickland, G. T., Lavanchy, D., Arthur, R. R., Magder, L. S., & Sallam, I. (2000). The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. The Lancet, 355(9207), 887-891.
11. Afzal, M.S. (2017) Hepatitis C Virus and Interferon-Free Antiviral Therapeutics Revolution: Implications for Pakistan. Viral Immunology. 30(4):252-257. doi: 10.1089/vim.2016.0164.
12. Afzal, M.S., Iqbal, M.A. (2017) Hepatitis C Virus in Pakistan: Community Education Is an Effective Weapon Against the Killer. Viral Immunol. 30(8):548-551 doi: 10.1089/vim.2016.0171.
13. Afzal, M.S., Khan, M.Y., Ammar, M., Anjum, S., Zaidi, N.U. (2014) Diagnostically untypable hepatitis C virus variants: it is time to resolve the problem. World J Gastroenterol. 20(46):17690-2. doi: 10.3748/wjg.v20.i46.17690.
14. Afzal, M.S., Shah, Z.H., Ahmed, H. (2016) Recent HCV genotype changing pattern in the Khyber Pakhtunkhwa province of Pakistan; is it pointing out a forthcoming problem? Braz J Infect Dis. 20(3):312-3. doi: 10.1016/j.bjid.2015.12.011.
15. Raza, H., Ahmad, T., Afzal, M.S. (2015) .HCV, Interferon Therapy Response, Direct Acting Antiviral Therapy Revolution and Pakistan: Future Perspectives. Asian Pac J Cancer Prev. 16(13):5583-4.
16. Khaliq, A., Ahmed, I., Afzal, M.S. (2018). HCV Treatment with Sofosbuvir in Pakistan; Current Scenario and Future Perspective. Journal of GHR. 7(2): 1-3. doi: 10.17554/j.issn.2224-3992.2018.07.770
17. Pape, G.R., Gerlach, T.J., Diepolder, H.M., Gruner, N., Jung, M.C., Santantonio, T. (1999) Role of the specific T-cell response for clearance and control of hepatitis C virus. J Viral Hepat 6: 36–40. pmid:10760033
18. Rehermann, B. (2013). Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. Nature medicine, 19(7), 859.
19. Ball, J.K., Tarr, A.W., McKeating, J.A. (2014). The past, present and future of neutralizing antibodies for hepatitis C virus. Antiviral research, 105, 100-111.
20. Edwards, V.C., Tarr, A.W., Urbanowicz, R.A., Ball, J.K. (2012). The role of neutralizing antibodies in hepatitis C virus infection. Journal of general virology, 93(1), 1-19.
21. Bailey JR, Wasilewski LN, Snider AE, El-Diwany R, Osburn WO, Keck Z, Foung SK, Ray SC (2015). Naturally selected hepatitis C virus polymorphisms confer broad neutralizing antibody resistance. J. Clin. Invest. , 125:437-447
22. Von Hahn T, Yoon JC, Alter H, Rice CM, Rehermann B, Balfe P, McKeating JA (2007). Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. Gastroenterology, 132:667-678. 3.
23. Swadling, P., Klenerman, Barnes, E. (2013) “Ever closer to aprophylactic vaccine for HCV,” Expert Opinion on Biological Therapy, 13 (8). 1109–1124.
24. Nguyen, C.T., and Tran, T.T. (2009) “Hepatitis vaccination and prophylaxis,” Clinics in Liver Disease, 13 (2); 317–329.
25. Linden bach BD, and Rice, C. M. (2013). The ins and outs of hepatitis C virus entry and assembly. Nat. Rev. Microbiol. 11, 688–700.
26. Freedman H, Logan MR, Law JLM, Houghton M. (2016). Structure and function of the hepatitis C virus envelope glycoproteins E1 and E2: antiviral and vaccine targets. ACS infectious diseases 2(11), 749-762.
27. Houghton, M. (2011). Prospects for prophylactic and therapeutic vaccines against the hepatitis C viruses. -Immunol. Rev. 239, 99–108.
28. Bui HH, Sidney J, Li W, Fusseder N, Sette A. (2007). Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines. BMC Bioinformatics, 8:361.
29. Idrees, S., & Ashfaq, U.A. (2013). Structural analysis and epitope prediction of HCV E1 protein isolated in Pakistan: an in-silico approach. Virology journal, 10(1), 113.
30. Arashkia, A., Roohvand, F., Memarnejadian, A., Aghasadeghi, M.R., Rafati, S. (2010) Construction of HCV-polytope vaccine candidates harbouring immune-enhancer sequences and primary evaluation of their immunogenicity in BALB/c mice. Virus Genes. 40(1):44–52.

ORIGINAL ARTICLE

PREDICTORS OF GASTRIC VARICES IN LIVER CIRRHOSIS PATIENTS

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ABSTRACT

Objective:
To determine different types of gastric varices and to find the predictors for development of gastric varices in patients suffering liver cirrhosis who underwent upper gastrointestinal endoscopy (UGIE) at Liver Clinic, Lahore (Pakistan).

Methodology:
This retrospective cohort study was done an liver cirrhosis patients who underwent UGIE from July 2011 to June 2014 were included. Gastric varices were divided into 4 types keeping in view the Sarin classification. Presence and types of gastric varices, gender, presence and grade of esophageal disease, presence of gastric vascular ectasia (GVE), and hiatal hernia were the qualitative variables, while age and weight of the patients were the quantitative variables. The data was evaluated on SPSS version 25. Means and standard deviations were computed for quantitative variable, and frequencies and percentages for qualitative variables. Independent sample t-test was applied to compare the mean value of age and weight of the patients with or without gastric varices. Chi-square test was applied to find associations of different qualitative variables with presence of gastric varices. Moreover, binary logistic regression analysis was also performed to find the significant predictors of gastric varices in liver cirrhosis patients. A p-value of equal to or less than 0.05 was considered as significant.

Results:
Out total of 2262 liver cirrhosis patients who underwent UGIE, 12.7% had gastric varices. Among different types of gastric varices, isolated gastric varix type 1 (IGV1) were the most prevalent (56.8%). Independent sample T-test found no significant difference of age (p=0.997) and weight (p=0.741) in patients with and without gastric varices. Bivariate analysis suggested that finding gastric varices had positive association with large size grade3 esophageal varices (P=0.01), and negative association with hiatal hernia (p=0.003) and gastric vascular ectasia (p=0.017). Logistic regression analysis also validated these findings.

Conclusion:
Isolated gastric varices type 1 were the most prevalent gastric varices in our liver cirrhosis patients. Presence of large size esophageal varices predict the presence of gastric varices. A significant inhibitory effect of hiatal hernia and gastric vascular ectasia was observed on the development of gastric varices in our studied population.

Key Words:
Gastric varices, Sarin classification, Predictors, Retrospective analysis

INTRODUCTION

Gastric varices (GV)[1] are the causative for life-threatening upper gastrointestinal bleed.[2] These are the dilated submucosal veins, which results from the elevated portal pressure or obstruction of the splenic vein.[3] According to Sarin, these are classified into 4 groups.[4] Gastroesophageal varices (GOV) type 1 are those esophageal varices which extend below cardia into stomach along lesser curvature, while GOV type 2 are those esophageal varices that extend below cardia into stomach along greater curvature. Isolated gastric varices (IGV) type 1 and IGV type 2 are defined by their presence in the fundus and elsewhere in the stomach respectively. The prevalence of GV in cirrhotic patients is 15-17%,[4-6] among which 75% are GOV1. The bleeding from GV has a very high mortality rate, approximately of 45%.[4] Type of GV (IGV1>GOV2>GOV1), large size, presence of red sign, and severity of liver dysfunction are the factors associated with higher risk of bleeding.[7] While looking gastric varices in liver cirrhosis patients during endoscopic examination, additional endoscopic findings like esophageal varices (EV), gastric vascular ectasia (GVE), and hiatal hernia are also noted. Whether these additional finding have any association, facilitatory or inhibitory effect on the development of gastric varices, national and international data is scarce. The objective of this study was to determine different types of gastric varices and to find the predictors for development of gastric varices in patients suffering Liver cirrhosis who underwent upper gastrointestinal endoscopy (UGIE) at Liver Clinic, Lahore, Pakistan.

METHODOLOGY

This was a retrospective cohort analysis[8] which was performed at Liver clinic, 250 Shadman Lahore. Amongst liver cirrhosis patients who underwent UGIE from July 2011 to June 2014, the patients with gastric varices were

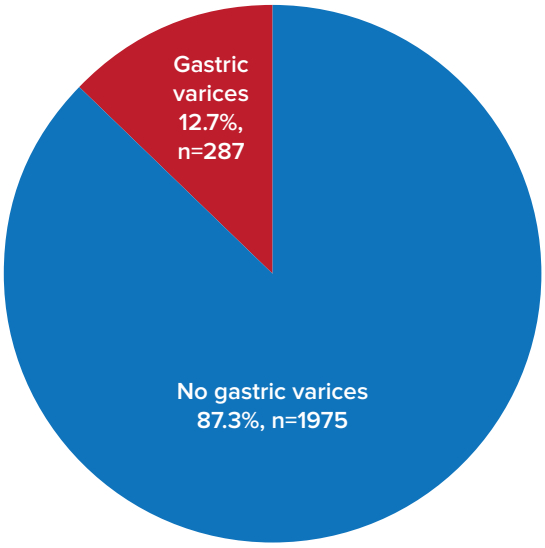

Figure 1: Prevalence of gastric varices among patients who underwent Upper GI endoscopy (n=287/2262)

included. GV were divided into 4 types keeping in view the Sarin classification.[3] The esophageal varices extending below the cardia into stomach along lesser curvature and greater curvature were named as GOV1 and GOV2 respectively, while isolated gastric varices in the fundus and elsewhere in the stomach were named as IGV1 and IGV2 respectively. EV were graded from I to III grades: Small and straight EV were Grade I, EV, tortuous varices occupying <1/3 of the esophageal lumen were grade II, and larger occupying >1/3 of the esophageal lumen were grade III EV.[9]

The presence gastric varices, types of gastric varices, gender, presence and grade of esophageal disease, presence of GVE, and hiatal hernia were the qualitative variables, while age and weight of the patients were the quantitative variables. The entire data was evaluated on SPSS version 25. Means and standard deviations were computed for quantitative variable, and frequencies and percentages for qualitative variables. Independent sample t-test[10] was applied to compare the mean value of age and weight of the patients with or without gastric varices. Chi-square test[11] was applied to find associations of different qualitative variables with presence of gastric varices. Moreover, binary logistic regression analysis was also performed to find the significant predictors of gastric varices in liver cirrhosis patients. A p-value of equal to or less than 0.05 was considered as significant.

Table 1: Prevalence of different types of gastric varices among patients who underwent upper GI endoscopy (n=287/2262)

Type of Gastric Varices	Frequency (Percent)
Isolated Gastric Varix 1 (IGV1)	163 (56.8%)
Gastroesophageal Varix 1 (GOV1)	68 (23.7%)
Gastroesophageal Varix 2 (GOV2)	38 (13.2%)
GOV1 + IGV1	13 (4.5%)
Isolated Gastric Varix 2 (IGV2)	3 (1.0%)
GOV1 + GOV2	2 (0.7%)

RESULTS

A total of 2262 liver cirrhosis patients underwent upper GI endoscopy, out of which 12.7% (n=287) had gastric varices **(figure 1)**. Among different types of gastric varices, isolated gastric varix 1 (IGV1) were most prevalent (56.8%). Other gastric varices found were gastroesophageal varix 1 (23.7%), gastroesophageal varix 2 (13.2%), and isolated gastric varix 2 (1%) **(table 1)**. The mean age of the patients who had gastric varices was 51.11 + 11.08 years and the mean age of the patients who had no gastric varices was 51.11 + 10.19 years. There was no statistically significant difference between the mean ages of the patients of two groups (p=0.997) **(table 2)**. Similarly, the mean weight of the patients who had gastric varices was 72.83 + 14.86 kilogram and the mean weight of the patients who had no gastric varices was 72.50 + 15.64 years. There was no statistically significant difference between the mean weight of the patients of two groups (p=0.741) **(table 3)**. Bivariate analysis suggested that large size grade3 esophageal varices had positive association with finding gastric varices on UGIE (P=0.01), while hiatal hernia and gastric vascular ectasia had negative association with gastric varices presence (p=0.003, p=0.017 respectively). There was no statistically significant association of gender (p=0.684) and presence/absence of gastric varices (p=0.754) with finding gastric varices during endoscopic examination among liver cirrhosis patients **(table 4)**.

A logistic regression was performed to ascertain the effect of age, weight, gender, presence of esophageal varices, presence of large size esophageal varices, hiatal hernia, and gastric vascular ectasia on the likelihood that gastric varix would be the finding of the upper GI endoscopy in liver cirrhosis patients. The logistic regression model was statistically significant, p<0.05. The model explained 2.6% (Nagelkerke R2) of the variance in group of patients

Table 2: Comparison of mean age of patients with gastric varices (present/absent) found during upper GI endoscopic examination (n=2262)*

Gastric Varices	Mean Age (Years)	Standard deviation	Mean difference	p-value	95% Confidence interval
Yes	51.11	11.08	-0.002	0.997	-1.279 – 1.274
No	51.11	10.19			

*Independent sample T-test was used

Table 3: Comparison of mean weight of patients with gastric varices (present/absent) found during upper GI endoscopic examination (n=2262)*

Gastric Varices	Mean Age (Years)	Standard deviation	Mean difference	p-value	95% Confidence interval
Yes	51.11	11.08	-0.002	0.997	-1.279 – 1.274
No	51.11	10.19			

*Independent sample T-test was used

who had gastric varices and correctly classified 87.3% of cases. Presence of large size grade3 esophageal varices significantly increases the likelihood of finding gastric varices, while presence of hiatal hernia and GVE significantly decreases the likelihood of finding gastric varices during upper GI endoscopic examination in liver cirrhosis patients **(table 5)**.

DISCUSSION

Different types of gastric varices have different locations and management plans, endoscopist should be familial of these facts because when these varices bleed in decompensated liver cirrhosis patient, it usually prove fatal. Esophageal varices and GOV1 form from a branch of portal vein, known as left gastric vein, while branches of splenic vein, posterior gastric vein and short gastric vein are involved in the formation of GOV2 and IGV1. Some branches of splenic vein and superior mesenteric vein are responsible for IGV2.[12] Similarly, veins draining these varices into vena cava also differ.[13] GOV1 and esophageal varices share the vascular anatomy, so treatment plan for both is same. Primary prophylaxis includes non-selective beta blockers (NSBB) for high risk small EV/GOV1 (with red sign or in CTP-C patient), and NSBB or carvedilol, or endoscopic variceal band ligation (EVBL) for medium or large EV/GOV1. Secondary prophylaxis includes combination of NSBB and EVBL (1st line) and TIPS (2nd line). For GOV2 or IGV1, primary prophylaxis includes NSBB while secondary prophylaxis includes TIPS or BRTO. Similarly, for actively bleeding GOV2 or IGV1, TIPS is the treatment of choice. Cyanoacrylate glue injection is an option if TIPS or BRTO are not technically feasible in secondary prophylaxis or actively bleeding GOV2 or IGV1.[14-16] Dual venous connection makes the management of GOV 2 difficult. BRTO eradicates its posterior (fundal) part drained by IPV and may remain its anteromedial (cardiac) part which is drained by esophageal varices. This part may need transhepatic embolization or EVBL.[17] Mudawi, Ali and Tahir[6] reported 16% prevalence of gastric varices in their study. Similarly, in 2007, Khalid Mumtaz

Table 4: Association of different qualitative parameters with finding of gastric varices during upper GI endoscopic examination (n=287/2262)*

Parameters/Categories	Gastric Varices		Total	p-value
	Yes	No		
1. Gender:				
Male	188 (65.5%)	1266 (64.1%)	1455	0.684
Female	99 (34.5%)	708 (35.9%)	807	
2. Esophageal Varices:				
Present	266 (92.7%)	1851 (93.8%)	2118	0.754
Absent	21 (7.3%)	123 (6.2%)	144	
3. Large Size (Grade 3) Esophageal Varices:				
Yes	109 (38%)	597 (30.2%)	707	0.010
No	178 (62%)	1377 (69.8%)	1555	
4. Hiatal Hernia:				
Yes	1 (0.3%)	90 (4.6%)	91	0.003
No	287 (99.7%)	1884 (95.4%)	2171	
5. Gastric Vascular Ectasia:				
Yes	14 (4.9%)	200 (10.1%)	214	0.017
No	273 (95.1%)	1774 (89.9%)	2048	

*Chi-square test for independence was used

Table 5: Binary Logistic Regression Output with Co-efficient, Odds Ratio and their 95% CI

Risk Factors	B	S.E.	Wald-Statistic	p-value	Odds Ratio	95% C.I. for EXP (B)	
						Lower	Upper
Age (Years)	0.001	0.006	0.019	0.890	1.001	0.989	1.013
Weight (Kilogram)	0.001	0.004	0.030	0.863	1.001	0.993	1.009
Gender (Male/Female)	0.049	0.139	0.125	0.723	1.050	0.800	1.379
Esophageal Varices (Yes/No)	-0.300	0.254	1.396	0.237	0.741	0.451	1.218
Large Size (Grade 3) Esophageal Varices (Yes/No)	0.282	0.136	4.315	0.038	1.326	1.016	1.729
Hiatal Hernia (Yes/No)	-2.503	1.009	6.152	0.013	0.082	0.011	0.591
Gastric Vascular Ectasia (Yes/No)	-0.703	0.286	6.044	0.014	0.495	0.283	0.867
Constant	-1.783	0.510	12.206	<0.01	0.168		

Nagelkerke R Square = 2.6%, Cox & Snell R Square = 1.4%

and his colleagues[5] from Karachi found the prevalence of GV of 15% in portal hypertension patients. In our study, liver cirrhosis patients, the prevalence of GV was 12.7%. The factors associated or responsible for this decreasing prevalence of GV in our liver cirrhosis patients require further studies with large sample size to validate these findings. In 1992, Sarin et al[1] found that GOV1 was the most common (75%) among all gastric varices. The also reported that IGV2 were extremely infrequent. In our study, IGV1 were most prevalent (56.8%), while IGV2 were the least prevalent (1.0%). This prevalence variation may be attributed to difference in distribution of portal hypertension in our population, which also warrant new studies focusing these points. Mudawi and colleagues also noted that GV were more prevalent in patients with grade I and grade II EV. However, reverse was found in our data. The prevalence of GV was 10.2%, 12.6%, and 15/1% in patients with grade I, II, and III EV respectively. Whether, gastric varices have protective role in the development of hiatal hernia in cirrhotic patients. International data is scarce on this hypothesis. In our data, 0.3% (1 out of 288) cirrhotic patients with gastric varices had hiatal hernia, while 4.6% (90 out of 1974) cirrhotic patients without gastric varices had hiatal hernia. In past, multiple studies showed high prevalence of GERD in liver cirrhosis patients.[18] No one study correlated hiatal hernia in cirrhosis patients with gastric varices. Our observation of less prevalence of hiatal hernia in conjunction with gastric varices may point the protective role of gastric varices in occurrence of hiatal hernia in cirrhotic patients. This hypothesis may require further studies to be validated. In our study, 4.9% (14 out of 287) cirrhotic patients with gastric varices had GVE while 10.1% (200 out of 1974) cirrhotic patients without gastric varices had GVE. Perhaps, development of gastric varices in cirrhotic patients has an inhibitory effect on development of GVE. No reference evidence was found in literature discussing this effect. I think, further larger studies are required to validate these findings.

CONCLUSION

Our study concluded that Intragastric varices type 1 were the most prevalent gastric varices in our liver cirrhosis patients. Presence of large size esophageal varices predict the presence of gastric varices as well. An inhibitory effect of hiatal hernia and gastric vascular ectasia was also observed on the development of gastric varices in our studied population.

REFERENCES

1. Sarin S. Gastric varices. In: Groszmann RJ, Bosch J, editors. Portal Hypertension in the 21st Century. Dordrecht: Springer; 2004: 277-300
2. Hot S, Yeşiltaş M, Gökçek B, Eğin S, Şengiz S. Massive and life-threatening upper gastrointestinal bleeding due to invasive hepatocellular carcinoma: A case report. Int J Surg Case Rep. 2016;26:69–72.
3. Malay Sharma M, Rameshbabu CS. Collateral Pathways in Portal Hypertension. J Clin Exp Hepatol 2012; 2(4): 338–352
4. Sarin SK, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. Hepatology 1992; 16(6): 1343-9.
5. Mumtaz K, Majid S, Shah HA, Hameed K, Ahmed A, et al. Prevalence of gastric varices and results of sclerotherapy with N-butyl 2 cyanoacrylate for controlling acute gastric variceal bleeding. World J Gastroenterol 2007; 13(8): 1247–1251.
6. Mudawi H, Ali Y, Tahir EM. Prevalence of gastric varices and portal hypertensive gastropathy in patients with Symmers periportal fibrosis. Ann Saudi Med 2008; 28(1): 42-4.
7. Kim T, Shijo H, Kokawa H, Tokumitsu H, Kubara K, Ota K, et al. Risk factors for hemorrhage from gastric fundal varices. Hepatology 1997; 25: 307-312.
8. Klebanoff MA, and Snowden JM. Historical (retrospective) cohort studies and other epidemiologic

study designs in perinatal research. Am J Obstet Gynecol. 2018;219(5):447-450.
9. Ferguson JW, Tripathi D, Hayes PC. Endoscopic diagnosis, grading and predictors of bleeding in esophageal and gastric varices. Techniques in Gastrointestinal Endoscopy 2005; 7 (1): 2-7. https://doi.org/10.1016/j.tgie.2004.10.002
10. Kim TK. T test as a parametric statistic. Korean J Anesthesiol. 2015;68(6):540–546. doi:10.4097/kjae.2015.68.6.540.
11. Pandis N. The chi-square test. American J of Orthod and Dentofacial Orthop.2016; 150(5):898 – 899.
12. Arakawa M, Masuzaki T, Okuda K. Pathomorphology of esophageal and gastric varices. Semin Liver Dis. 2002; 22: 73–82.
13. Moubarak E, Bouvier A, Boursier J, Lebigot J, Ridereau-Zins C, et al. Portosystemic collateral vessels in liver cirrhosis: a three-dimensional MDCT pictorial review. Abdom Imaging. 2012; 37(5): 746-66.
14. Lo GH, Liang HL, Chen WC, Chen MH, Lai KH, Hsu PI, et al. A prospective, randomized controlled trial of transjugular intrahepatic portosystemic shunt versus cyanoacrylate injection in the prevention of gastric variceal rebleeding. Endoscopy 2007; 39: 679-685.
15. Fukuda T, Hirota S, Sugimura K. Long-term results of balloon-occluded retrograde transvenous obliteration for the treatment of gastric varices and hepatic encephalopathy. J Vasc Interv Radiol 2001; 12: 327-336.
16. Garcia-Tsao G, Abraldes J, Berzigotti A, and Bosch J. Portal Hypertensive Bleeding in Cirrhosis: Risk Stratification, Diagnosis, and Management: AASLD Guidelines 2016. Hepatology 2017; 65 (1): 310-335.
17. Uchiyama F, Murata S, Onozawa S, Nakazawa K, Sugihara F, et al. Management of Gastric Varices Unsuccessfully Treated by Balloon-Occluded Retrograde Transvenous Obliteration: Long-Term Follow-Up and Outcomes. The Scientific World Journal 2013; 1: 1-7
18. Ahmed AM, al Karawi MA, Shariq S, Mohamed AE. Frequency of gastroesophageal reflux in patients with liver cirrhosis. Hepatogastroenterology 1993; 40: 478-480 [PMID: 8270239]

ORIGINAL ARTICLE

PREVALENCE & ASSOCIATIONS OF ANORECTAL DISORDERS AMONG PATIENTS WHO UNDERWENT SIGMOIDOSCOPY

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ABSTRACT

Objective:
To determine the prevalence of anorectal disorders among patients who underwent Sigmoidoscopy at Liver Clinic, Lahore, Pakistan. In addition, our study will also determine the association of finding anorectal disease with gender and age in our studied population.

Methodology:
In this retrospective analysis of patients who underwent sigmoidoscopy, presenting symptoms were grouped into per-rectal (PR) bleed, painful defecation, anal discomfort, and altered bowel habits (ABH). The anorectal diagnoses made during sigmoidoscopy were noted. The gender, presenting symptom, and endoscopic diagnosis were the qualitative variables, while age of the patients was the only quantitative variable. The data was analyzed using SPSS version 15. The means and standard deviations were computed for quantitative variable, and frequencies and percentages for qualitative variables. Association of age with finding anorectal disease on sigmoidoscopy was performed using Independent sample T-test while association of gender with finding the anorectal disease was performed using chi-square test of Independence. A p-value equal or less than 0.05 was considered statistically significant.

Results:
Out of the total of 935 patients, 52.2% were diagnosed with anorectal disease. The presenting symptoms of patients diagnosed with anorectal disease were per-rectal (PR) bleed (47.2%, n=232), painful defecation (11.4%, n=56), and anal discomfort (0.6%, n=3). 40.9% (n=201) patients had no anorectal complaint, rather anorectal disease was accidental finding. The percentage (frequency) distribution of anorectal diseases like internal hemorrhoids, anal fissure, tight sphincter without visible fissure, external hemorrhoids, anal stenosis, fibrotic patch at anorectal junction, anal skin tags, foreign body, 3rd degree perineal tear was 90.0% (n=317), 5.9% (n=21), 0.3% (n=1), 2.2 % (n=8), 0.3% (n=1), 0.8% (n=3), 0% (n=0), 0.2% (n=1), and, 0% (n=0) respectively in male gender while 82.8% (n=130), 7.0% (n=11), 4.4% (n=7), 2.6 % (n=4), 1.9% (n=3), 0% (n=0), 0.6% (n=1), 0% (n=0), and, 0.6% (n=1) respectively in female gender. The mean age of patients diagnosed with anorectal disease was significantly higher than patients with no anorectal disease (45.76 + 13.43 years vs 41.41 + 16.83 years, p<0.01). The finding anorectal disease on sigmoidoscopic examination had no statistically significant association with gender (p=0.684).

Conclusion:
Anorectal disease is a prevalent finding during sigmoidoscopic examination, where internal hemorrhoid and anal fissure are dominantly more common in male gender while tight sphincter without visible fissure and anal stenosis in female gender group. PR bleed was presenting symptom of majority patients; however, in a big proportion of patients, anorectal disorder was an accidental finding in which the procedure was done for other symptoms like altered bowel habit. Finding an anorectal disease on sigmoidoscopy had a positive association with increasing age and no association with gender.

Key Words:
Anorectum, Sigmoidoscopy, Prevalence, Retrospective analysis, SPSS

INTRODUCTION

The anorectum is the distal part of gastrointestinal tract which includes anal canal and distal 2cm of adjacent rectum.[1,2] It develops from fusion of rectum with anal canal at 8th week of gestation when anal membrane ruptures.[3] The dentate line points the line of fusion. Anal canal is 3-4cm long and extends from this dentate line till anal verge.[4] It has two sphincters lying distally. External sphincter is composed of voluntary striated muscles, while internal sphincter is a continuation of involuntary circular muscles of rectum.[5] Anorectum is affected by spectrum of specialized diseases[6] which include hemorrhoids, anal fissure, unexplained anal pain, anal stenosis, anal warts, anal skin tags, anal malignancy, perianal fistulas and abscesses. Internal hemorrhoid is the commonest anorectal disorder,[7] where per-rectum (PR) bleed is the usual presenting symptom. Pelvic floor disorders affect 10–15% of the general population.[8,9]

Flexible sigmoidoscopy[10] is the direct visualization of distal gut from anal verge till splenic flexure. It is superior than Hirschman anoscopy for anorectal examination, where therapeutic maneuvers like hemorrhoidal rubber band ligation can be offered in addition to diagnosis. In comparison to colonoscopy, flexible sigmoidoscopy does

not require full colonic preparation; only purgation with enema performed preprocedural is enough.[11] The data on the prevalence of anorectal diseases, their variety and types and statistical associations is scarce. Therefore, the objective of our study was to determine the prevalence of anorectal disorders among patients who underwent Sigmoidoscopy at Liver Clinic, Lahore, Pakistan. In addition, our study will also determine the association of finding anorectal disease with gender and age in our studied population.

METHODOLOGY

This retrospective analysis[12] included all those patients who underwent sigmoidoscopy from February 2011 to July 2017 at Liver clinic, 250 Shadman Lahore. The presenting symptoms were grouped into per-rectal (PR) bleed, painful defecation, anal discomfort, and altered bowel habits (ABH). Large bowel diarrhea, constipation, PR mucous, abdominal distension with inadequate evacuation were grouped as ABH. The anorectal diagnoses were noted. The gender, presenting symptom, and endoscopic diagnosis were the qualitative variables, while age of the patients was the only quantitative variable. The data analysis was performed using SPSS version 15. The means and standard deviations were computed for quantitative variable, and frequencies and percentages for qualitative variables. Association of age with finding anorectal disease on sigmoidoscopy was performed using Independent sample T-test[13] while association of gender with finding the anorectal disease was performed using chi-square test of Independence.[14] A p-value equal or less than 0.05 was considered statistically significant.

RESULTS

A total of 935 patients underwent sigmoidoscopy, out of which 492 (52.2%) were diagnosed with anorectal disease **(figure 1)**. Among patients who diagnosed with anorectal disease, presenting symptoms were per-rectal

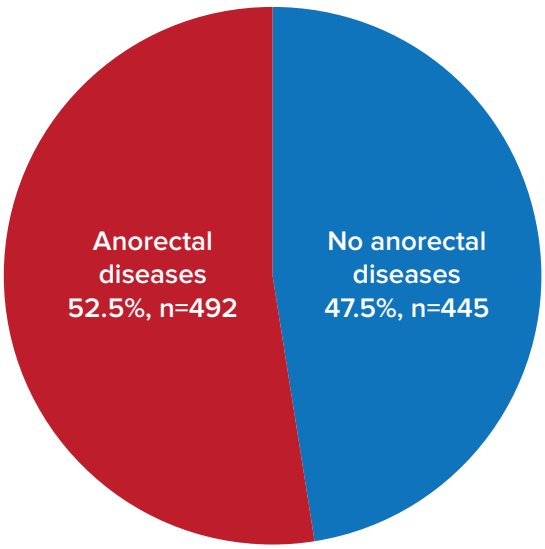

Figure 1: Prevalence of anorectal disease in patients who underwent sigmoidoscopy (n=937)

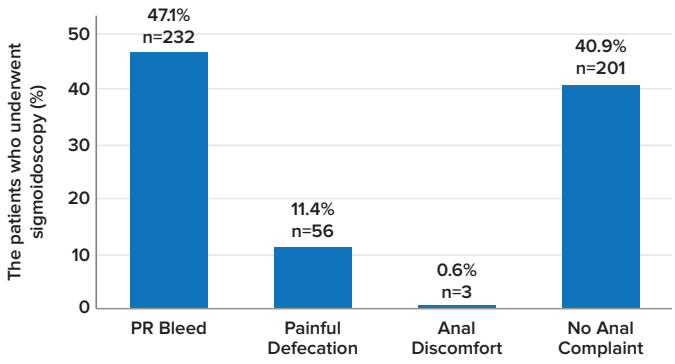

Figure 2: Spectrum of symptoms of patients who diagnosed with anorectal disease on sigmoidoscopy (n=492/937)

(PR) bleed (47.2%, n=232), painful defecation (11.4%, n=56), and anal discomfort (0.6%, n=3). 40.9% (n=201) patients had no anorectal complaint, rather anorectal disease was accidental finding during sigmoidoscopic examination for altered bowel habits **(figure 2)**.

The percentage (frequency) distribution of anorectal diseases like internal hemorrhoids, anal fissure, tight sphincter without visible fissure, external hemorrhoids, anal stenosis, fibrotic patch at anorectal junction, anal skin tags, foreign body, 3rd degree perineal tear was 90.0% (n=317), 5.9% (n=21), 0.3% (n=1), 2.2 % (n=8), 0.3% (n=1), 0.8% (n=3), 0% (n=0), 0.2% (n=1), and, 0% (n=0) respectively in male gender while 82.8% (n=130), 7.0% (n=11), 4.4% (n=7), 2.6 % (n=4), 1.9% (n=3), 0% (n=0), 0.6% (n=1), 0% (n=0), and, 0.6% (n=1) respectively in female gender **(table 1)**.

The mean age of patients diagnosed with anorectal disease was significantly higher than patients with no anorectal disease (45.76 + 13.43 years vs 41.41 + 16.83 years, p<0.01) **(table 2)**. The finding anorectal disease on sigmoidoscopic examination had no statistically significant association with gender (p=0.684) **(table 3)**.

Table 1: Gender wide distribution of different types of anorectal diseases (n=562/1004)

Diseases of Anorectum	Frequency (%) Among Male	Frequency (%) Among Female
Internal Hemorrhoids	317 (90.0%)	130 (82.8%)
Anal Fissure	21 (5.9%)	11 (7.0%)
Tight Sphincter, without Visible Fissure	1 (0.3%)	7 (4.4%)
External Hemorrhoids	8 (2.2%)	4 (2.6%)
Anal Stenosis	1 (0.3%)	3 (1.9%)
Fibrotic Patch at Anorectal Junction	3 (0.8%)	0 (0%)
Anal Skin Tags	0 (0%)	1 (0.6%)
Foreign Body	1 (0.2%)	0 (0%)
Perineal Tear, 3rd Degree	0 (0%)	1 (0.6%)

Table 2: Comparison of mean age in groups of patients with Anorectal disease (Yes/No) (n=937)[1]

Anorectal Disease	Mean Age (Years)	Standard Deviation	Mean Difference	p-value	95% Confidence Interval
Yes	45.76	13.43	4.35	<0.01	2.407 - 6.295
No	41.41	16.83			

1= Independent sample T-test was used

DISCUSSION

Anorectal disorders are common world-wide, where flexible sigmoidoscopy is excellent tool for their diagnosis and even management in majority cases.[15] In our study, prevalence of finding anorectal disorder was high among patients who underwent sigmoidoscopy. Even 40.9% (n=201) patients had no proctological symptoms and an anorectal disorder was found among them. In their study, Nelson NR and colleagues[16] also found very high prevalence of anorectal diseases in general population. Similarly, Laurent Abramowitz et al[17] found 14% prevalence of proctological symptoms in general population. In a western population study by Abubakr Ahmed and colleagues[18] on patients with proctological symptoms who underwent sigmoidoscopy, female participants were more than males (50.6% vs 49.4). In our study, among patients diagnosed with anorectal disorder, 1455 were male in contrast to 807 females. Social and cultural constraints make it difficult for our female to talk about their anal disorders, and our doctors do not always ask patients about potential proctological symptoms, which further delay diagnosis. Proctological teaching is generally very limited in Pakistan. This issue of our societies requires special attention.

In same study from Ireland,[18] PR-bleed (52.4%) was a leading symptom of patients undergoing sigmoidoscopic examination. Similarly, in our study of patients with diagnosed diseases of anorectum, PR bleed (47.1%) was the commonest symptom followed by painful defecation (11.4%), and anal discomfort (0.6%). In Laurent Abramowitz study,[17] bleeding (32%) was also the commonest proctological symptom followed by pain (31%), pruritis ani (22%) and anal discharge (14%). Phillip K. Henderson[7] found internal hemorrhoids and anal fissure as the most prevalent anorectal disorder. Similar were the findings in our study. Nelson et al[16] stated female gender as the best predictor of benign anorectal disease (odds ratio = 4.6; 95% confidence interval = 1.3-20.4). However, in our data, it was noted that gender has no association with finding an anorectal disorder on sigmoidoscopic examination (p=0.684). Siegfried W.B. Yu and SSC Rao[19] said that anorectal disorders such as chronic constipation, dyssynergic defecation, fecal impaction, and overflow fecal incontinence are highly prevalent in the elderly. ASGE (American Society for Gastrointestinal Endoscopy)[20] also found that both incidence and prevalence of anorectal disorders rises with increasing age. Similarly, our study showed higher mean age of the patients with anorectal disorders as compared to patients without those disorders. Further larger studies are required to validate these findings.

CONCLUSION

Anorectal disease is a prevalent finding during sigmoidoscopic examination, where internal hemorrhoid and anal fissure are dominantly more common in male gender while tight sphincter without visible fissure and anal stenosis in female gender group. PR bleed was presenting symptom of majority patients; however, in a big proportion of patients, anorectal disorder was an accidental finding in which the procedure was done for other symptoms like altered bowel habit. Finding an anorectal disease on sigmoidoscopy had a positive association with increasing age and no association with gender.

REFERENCES

1. Stoker J. Anorectal and pelvic floor anatomy. Best Pract Res Clin Gastroenterol. 2009;23:463–75.
2. http://medical-dictionary.thefreedictionary.com/anorectum.
3. Su P, Yuan Y, Huang Y, Wang W, Zhang Z. Anorectal malformation associated with a mutation in the P63 gene in a family with split hand-foot malformation. Int J Colorectal Dis. 2013;28(12):1621–1627.
4. Salati SA, Al Kadi A. Anal cancer - a review. Int J Health Sci (Qassim). 2012;6(2):206–230.
5. Agarwal S. Anatomy of the Pelvic Floor and Anal Sphincters. JIMSA 2012; 25(1): 19-21.
6. Foxx-Orenstein AE, Umar SB, Crowell MD. Common anorectal disorders. Gastroenterol Hepatol (N Y). 2014;10(5):294–301.
7. Henderson PK, Cash BD. Common anorectal conditions: Evaluation and treatment. Current

Table 3: Association of gender with finding anorectal disease during sigmoidoscopic examination (n=937)*

Gender	Anorectal Disease		Total	p-value
	Yes	No		
Male	188 (65.5%)	1266 (64.1%)	1455	0.684
Female	99 (34.5%)	708 (35.9%)	807	

*Chi-square test for independence was used

Gastroenterology report2 2014; 16: 408.
8. Bharucha AE, Zinsmeister AR, Locke GR, Seide B, McKeon K, Schleck CD, Melton LJI. Prevalence and burden of fecal incontinence: A population-based study in women. Gastroenterology. 2005; 129:42–49.
9. Bharucha AE, Locke GR, Pemberton JH. AGA Practice Guideline on Constipation: Technical Review. Gastroenterology. 2013; 144:218–238.
10. Adebogun AO, Berg CD, Laiyemo AO. Concerns and challenges in flexible sigmoidoscopy screening. Colorectal Cancer. 2012;1(4):309–319.
11. Manoucheri M, Nakamura DY, Lukman RL. Bowel preparation for flexible sigmoidoscopy: which method yields the best results? J Fam Pract 1999; 48(4): 272-4.
12. Hess DR. Retrospective studies and chart reviews. Respir Care. 2004;49(10):1171-4.
13. Sedgwick P. Independent samples t test. BMJ 2010;340:c2673.
14. McHugh ML. The chi-square test of independence. Biochem Med. 2013;23(2):143–149.
15. Appalaneni V, Fanelli RD, Sharaf RN, Anderson MA, Banerjee S, Ben-Menachem T. The role of endoscopy in patients with anorectal disorders. Gastrointestinal Endoscopy 2010; 72(6): 1117 – 1123.
16. Nelson RL, Abcarian H, Davis FG, Persky V. Prevalence of benign anorectal disease in a randomly selected population. Dis Colon Rectum 1995;38(4):341-4.
17. Abramowitz L, Benabderrahmane M, Pospait D, Philip J, & Laouénan C. The prevalence of proctological symptoms amongst patients who see general practitioners in France. European Journal of General Practice 2014; 20:4:301-30.
18. Ahmed A, Sadadcharam G, Lynch N, Myers E, Andrews E. Diagnostic yield of flexible sigmoidoscopy in symptomatic population: An insite to rapid access sigmoidoscopy clinic. Surg Res Open J 2014; 1(1):10-16.
19. Yu SW, Rao SS. Anorectal physiology and pathophysiology in the elderly. Clin Geriatr Med. 2014;30(1):95–106.
20. ASGE (American Society for Gastrointestinal Endoscopy) guidelines. The role of endoscopy in patients with anorectal disorders. Gastrointestinal endoscopy 2010;72(6): 1117-23.

ORIGINAL ARTICLE

PREVALENCE OF LOWER GASTROINTESTIONAL SYMTOMS IN DIFFERENT AGE GROUPS: A DESCRIPTIVE ANALYSIS OF PATIENTS WHO HAD SIGMOIDOSCOPIC EXAMINATION

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ABSTRACT

Objective:
To determine the revalence of various lower gastrointestional symtoms in different age groups amongst patients who underwent sigmoidoscopy at Liver Clinic, Lahore (Pakistan).

Methodology:
It was a cross sectional study. Using consecutive sampling technique, the data of all the patients who had sigmoidoscopy from July 2010 to June 2014 was collected from computer data base. The chief presenting symptoms were noted. Age of the patients was categorized into 5 groups: group 1 (childhood: age <13 years), group 2 (adolescence: age 13-18 years), group 3 (young adults: age 19-44 years), group 4 (middle aged adults: age 45-65 years), and group 5 (older adults: age >65 years). The data was analyzed using SPSS version 25.

Results:
Out of the total of 528 patients, 0.9% were children, 3.2% adolescents, 52.1% young adults, 37.5% middle aged adults, and 6.3% were older adults. 46.4%patients had altered bowel habit (ABH), 44.3% per-rectal (PR) bleed, 7.8% painful defecation, and 1.5% patients had anal discomfort as chief presenting symptom. The prevalence of PR bleed was 20%, 23.5%, 48.4%, 43.4%, and 63.6% in age group 1 to 5 respectively. This accelerating pattern of prevalence of PR bleed with increasing age was statistically significant (p=0.04). ABH was most prevalent in children (80%), anal discomfort in adolescents (5.9%), and painful defecation in middle aged adults (11.1%).

Conclusion:
Young adulthood was the commonest age group among the patients who underwent sigmoidoscopy. The prevalence of PR bleed increased in incremental pattern with increasing age group. ABH was most prevalent in children, anal discomfort in adolescents, and painful defecation in middle aged adults amongst our studied population.

Key Words:
Lower GI complaints, Age groups, Sigmoidoscopy, SPSS

INTRODUCTION

Gastrointestinal (GI) symptoms[1] are common in the community and carry heavy social and economic consequences.[2] Broadly, these can be divided into upper and lower GI complaints.[3] Lower GI complaints are multiple including per-rectal (PR) bleed,[4] diarrhea,[5] constipation,[6] fecal incontinence,[7] anal discomfort,[8] painful defecation.[9] Internal hemorrhoids, inflammatory bowel disease, infectious colitis, and polyps are the major identities which presents with PR bleed while anal fissure, anal stenosis, and worm infestation are the common causes for anal pain or discomfort. Altered bowel habit (ABH)[10] is a vague term and includes a change in frequency or consistency of stool i.e. diarrhea or constipation or both alternating. Internationally, age is categorized into different groups.[11,12] Age less than 13 years defines childhood while age between 13 to 18 years is labelled as adolescence. People with age 19 years or above are adults, where of 19-44 years are called young adults, 45-65 years are middle aged adults, and more than 65 years are older adults. Sigmoidoscopy[13] is a procedure to look inside distal portion of digestive tract from anus till splenic flexure. It is a walk-in-clinic type procedure, where full colonic preparation is not required; only pre-procedure purgation with enemas is enough.[14] What type of lower GI complaints were present among our patients who underwent sigmoidoscopy. Whether specific age group was keener to undergo sigmoidoscopy, and what types of lower GI complaints were prevalent in different age groups. All these questions made the author prudent to analyze the data retrospectively available at the computer database of Liver Clinic Lahore, Pakistan.

METHODOLOGY

Data for all patients who had flexible sigmoidoscopy between 1st July 2010 and 30th June 2014 was collected retrospectively from the computer database of the

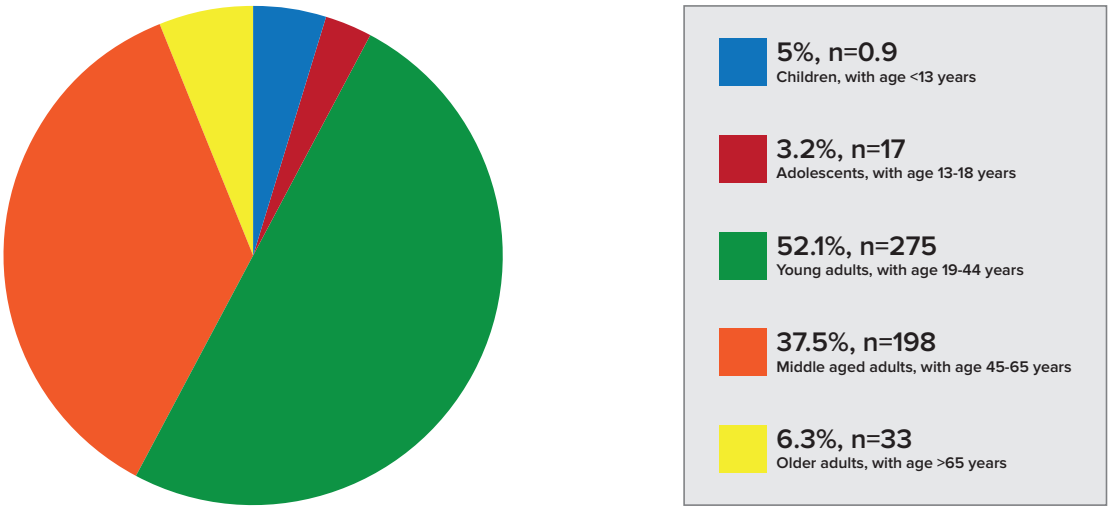

Figure 1: Different age groups of patients who underwent sigmoidoscopy (n=528)

endoscopy department of Liver clinic, 250 Shadman Lahore. Exclusion criteria included all those patients with endoscopic procedures other than sigmoidoscopy and incomplete data entry. After exclusion, the age of the patients was categorized into 5 groups: group 1 (childhood: age <13 years), group 2 (adolescence: age 13-18 years), group 3 (young adults: age 19-44 years), group 4 (middle aged adults: age 45-65 years), and group 5 (older adults: age >65 years).[11,12] Altered bowel habits (ABH), per-rectal (PR) bleed, anal discomfort, and painful defecation were noted as the presenting complaints for which sigmoidoscopy was performed. Large bowel diarrhea, constipation, PR mucous, abdominal distension with inadequate evacuation was named as ABH. The gender, chief presenting complaint, and age groups were the qualitative variables, while age of the patients was the only quantitative variable. Statistical analysis was done using the Statistical Package for Social Science (SPSS), version 25. Frequencies and percentages were computed for qualitative variables, while mean and standard deviation were calculated for quantitative variables. The chi-square test of independence was applied on the data and p-values were considered as statistically significant if < 0.05.

RESULTS

A total of 528 patients had flexible sigmoidoscopy during this four year period; majority were male (68.9%, n=364). Their age ranged from 7-95years, with a mean value of 42.43 + 15.09. The frequencies and percentages of patients in different age groups were as follow: 5 (0.9%) patients in group 1, 17 (3.2%) patients in group 2, 275 (52.1%) patients in group 3, 198 (37.5%) patients in group 4, and 33 (6.3%) patients in group 5 **(figure 1)**. The frequencies and percentages of different lower GI symptoms were as follow: 245 (46.4%) patients had ABH, 234 (44.3%) patients had PR bleed, 41 (7.8%) patients had painful defecation, and 8 (1.5%) patients had anal discomfort **(figure 2)**.

The prevalence of PR bleed was 20% (in 1 out of 5 patients), 23.5% (in 4 out of 17 patients), 48.4% (in 133 out of 275 patients), 43.4% (in 86 out of 198 patients), and 63.6% (in 21 out of 33 patients) in age groups 1, 2, 3, 4, and 5 respectively. This accelerating pattern of prevalence of PR bleed as lower GI complaint with increasing age was statistically significant (p=0.04). The prevalence of ABH was 80%, 64.7%, 43.6%, 44.9%, and 36.4% in age group 1, 2, 3, 4, and 5 respectively. The prevalence of ABH has no statistically significant association with age groups (p=0.086). The prevalence of anal discomfort as lower GI complaints was 0%, 5.9%, 2.2%, 0.5%, and 0% in age group 1, 2, 3, 4, and 5 respectively. The prevalence of anal discomfort has no statistically significant association with age groups (p=0.295). The prevalence of painful defecation was 0%, 5.9%, 5.8%, 11.1%, and 6.1% in age group 1, 2, 3, 4, and 5 respectively. The prevalence of anal discomfort has no statistically significant association with age groups (p=0.295) **(table 1)**.

DISCUSSION

Rectal bleed, altered bowel habits, anal discomfort, and painful defecation are the symptoms for which majority patients undergo sigmoidoscopic examination. In 1987, Murray flotre[15] found rectal bleeding and abdominal pain

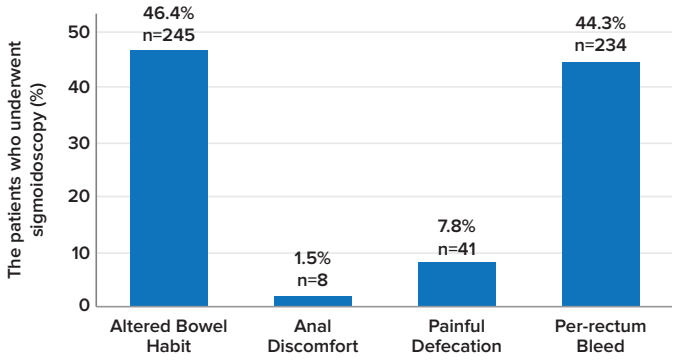

Figure 2: Different lower GI complaints among patients who underwent sigmoidoscopy (n=528)

Table 1: Correlation of lower GI complaints with age groups (n=528)

Lower GI Complaints	Age Groups (Frequency/Percent)					p-value
	1	2	3	4	5	
Per-rectum Bleed:						
Yes	1 (20%)	4 (23.5%)	133 (48.4%)	86 (43.4%)	21 (63.6%)	0.040
No	4 (80%)	13 (76.5%)	142 (51.6%)	112 (56.6%)	12 (36.4%)	
Altered Bowel Habit:						
Yes	4 (80%)	11 (64.7%)	120 (43.6%)	89 (44.9%)	10 (30.3%)	0.086
No	1 (20%)	6 (35.3%)	155 (56.4%)	109 (55.1%)	23 (69.7%)	
Anal Discomfort:						
Yes	0 (0.0%)	1 (5.9%)	6 (2.2%)	1 (0.5%)	0 (0.0%)	0.295
No	5 (100%)	16 (94.1%)	269 (97.8%)	197 (99.5%)	33 (100%)	
Painful Defecation:						
Yes	0 (0.0%)	1 (5.9%)	16 (5.8%)	22 (11.1%)	2 (6.1%)	0.268
No	5 (100%)	16 (94.1%)	259 (94.2%)	176 (88.9%)	31 (93.9%)	

1=Childhood; 2=Adolescence; 3=Young adults; 4=Middle aged adults; 5=Older adults

as the most common presenting complaints investigated with flexible sigmoidoscope, in 30.9% and 35.1% patients respectively. A similar study was performed in Ireland in 1914,[16] in which the commonest presenting symptom amongst patients referred for sigmoidoscopy was change in bowel habit accounting for 53.7% (n=239) patients followed by 52.4% of patients presenting with rectal bleeding (n=233). Similarly, in our study two major presenting complaints were ABH (46.4%) and rectal bleed (44.3%). In 445 patients' study of Abubakr Ahmed et al,16 average age of patients referred for sigmoidoscopy was 58.7 + 13.05 years. In our study, younger population underwent the procedure (mean age 42.43 + 15.09 years). 51.1% patients were young adults, 37.5% middle aged adults, and remaining only 10.4% were children, adolescents, and older adults. Longstreth GF[17] found that rate of hospitalization for acute lower GI hemorrhage increased >200 fold from the third to the ninth decades of life. According to Don C. Rockey,[18] the incidence of lower GI bleeding increases substantially with age, presumably due to the high incidence of diverticulosis and vascular disease in this group. Similarly, in our study, proportion of patients presenting with rectal bleeding increased as group of age moved from childhood to adolescence, till older adults i.e. as age increased. In our data of patients seeking sigmoidoscopic examination, 80% child, 64.7% adolescents, 43.6% young adults, 44.9% middle aged adults, and 36.4% older adults presented with altered bowel habits. A big proportion of patients presenting with ABH have IBS,[10,19] which occur in all age groups, including children[20] There is no difference seen in the frequency by age,[21] because of its lifelong relapsing and remitting pattern. Balamma Sujatha et al[22] found 13.5% prevalence of functional constipation in age group of 2-4years. Hence, all age groups can present with ABH, where sigmoidoscopy may be required to aid the diagnosis. Anal fissure is the commonest cause of painful defecation. Anal skin tag, thrombosed external hemorrhoids, anal comedones, worm infestation, and many more are the causes of anal discomfort. In our study, anal discomfort was more prevalent in adolescents, while painful defecation in middle aged adults. Further studies with large sample size are required to firmly validate these findings as well as to elaborate the etiologies for these complaints in our population.

CONCLUSION

In conclusion, young adulthood was the commonest age group among the patients who underwent sigmoidoscopy. Altered bowel habit, rectal bleed, painful defecation and anal discomfort were different lower GI complaints. The prevalence of PR bleed increased in incremental pattern with increasing age group. ABH was most prevalent in children, anal discomfort in adolescents, and painful defecation in middle aged adults amongst our studied population.

REFERENCES

1. Peery AF, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, et al. Burden of gastrointestinal diseases in united states: 2012 update. Gastroenterology 2012; 143 (5): 1179-1187.
2. Chang JY, Locke GR, McNally MA, Halder SL, Schleck CD, Zinsmeister AR, et al. Impact of Functional Gastrointestinal Disorders on Survival in the Community. Am J Gastroenterol 2010; 105(4): 822–832.
3. Talley NJ, Boyce P, and Jones M. Identification of distinct upper and lower gastrointestinal symptom

groupings in an urban population. Gut 1998; 42: 690–695.
4. Ellis BG, Thompson MR. Factors identifying higher risk rectal bleeding in general practice. Br J Gen Pract. 2005;55(521):949-55.
5. Sweetser S. Evaluating the patient with diarrhea: a case-based approach. Mayo Clin Proc 2012;87(6):596-602.
6. Portalatin M, Winstead N. Medical management of constipation. Clin Colon Rectal Surg 2012; 25(1): 12-9.
7. Wang JY, Abbas MA. Current management of fecal incontinence. Perm J. 2013; 17(3): 65-73.
8. https://www.iffgd.org/symptoms-causes/anal-discomfort.html
9. Sabatino MD, Borrelli M, Cautiero P, Romano M, Parmeggiani P, Marte A. Painful defecation and chronic functional constipation in children: Diagnosis and treatment. Minerva Pediatr 2006 Jun; 58(3): 299-304.
10. Holten KB and Bankston L. Diagnosing the Patient with Abdominal Pain and Altered Bowel Habits: Is It Irritable Bowel Syndrome?. Am Fam Physician 2003; 67(10): 2157-2162.
11. https://en.oxforddictionaries.com/definition/us/middle_age
12. www.widener.edu/about/campus_resources/wolfgram.../life_span_chart_final.pdf
13. LF Ashley, RL Jenifer, DA Ruben, A Deepak, HB David, et al. Guidelines for privileging, credentialing, and proctoring to perform GI endoscopy. Gastrointestinal endoscopy 2017; 85 (2): 273-281
14. Manoucheri M, Nakamura DY, Lukman RL. Bowel preparation for flexible sigmoidoscopy: which method yields the best results? J Fam Pract 1999; 48(4): 272-4.
15. Flotre M. Flexible Sigmoidoscopy in a Family Practice. Can Fam Physician 1987; 33: 2103-2106.
16. Ahmed A, Sadadcharam G, Lynch N, Myers E, Andrews E. Diagnostic yield of flexible sigmoidoscopy in symptomatic population: An insite to rapid access sigmoidoscopy clinic. Surg Res Open J. 2014; 1(1):10-16.
17. Longstreth GF. Epidemiology and outcome of patients hospitalized with acute lower gastrointestinal hemorrhage: a population-based study. Am J Gastroenterol 1997; 92(3): 419-24.
18. Rockey DC. Lower Gastrointestinal Bleeding. Gastroenterology 2006; 130: 165–171
19. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. Clin Epidemiol. 2014; 6: 71-80.
20. Rasquin A, Di Lorenzo C, Forbes D, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology. 2006;130(5):1527–1537.
21. Tang YR, Yang WW, Liang ML, Xu XY, Wang MF, Lin L. Age-related symptom and life quality changes in women with irritable bowel syndrome. World J Gastroenterol. 2012;18(48):7175–7183.
22. Sujatha B, Velayutham DR, Deivamani N, Bavanandam S. Normal Bowel Pattern in Children and Dietary and Other Precipitating Factors in Functional Constipation. J Clin Diagn Res. 2014; 3(2): 12-5.

ORIGINAL ARTICLE

RELATIONSHIP OF COAGULABILITY WITH FIBROSCAN MEASURED LIVER STIFFNESS INDEX (LSI) IN DIFFERENT STAGES OF FIBROSIS IN CHRONIC HEPATITIS C INFECTION

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ABSTRACT

Introduction:
In most cases, hepatitis C progresses gradually to chronic disease, frequently patient being unalarmed until progression to advanced fibrosis and cirrhotic stages. Clotting profile (PT, APTT) has been integral part of comprehensive assessment of patients presenting with liver impairment and they are among readily available liver function tests. We studied variation of PT, APTT with Fibroscan score in various stages of fibrosis in HCV infected patients.

Methods:
The retro-prospective cross sectional study was carried out at LGH, Lahore from February 14, 2018 to January 14, 2019. We studied on 260 HCV infected patients, patients having coagulopathy other than liver impairment were excluded. Patients were assessed for PT, APTT and fibrosis stage was determined by Fibroscan score. To determine the significant association between continuous variables and liver fibrosis stages, Spearman's rank correlation was used.

Results:
For stage F0-F1 and F2, PT and APTT variables showed non- significant relationship ($p > 0.05$) with fibroscan score or liver stiffness index, while for stage F3 and F4, significant relationship with fibroscan score or liver stiffness index was found with $p=0.022$ and $p=0.01$ respectively. Patients with F4 (cirrhotic) stage had second highest distribution.

Conclusion:
The study implicates that, using PT, APTT lab tests, it can be predicted whether the patient having chronic course of hepatitis C, has progressed to F3, cirrhosis (F4)

Key Words:
Hepatitis C, Prothrombin time, Activated partial prothrombin time, Coagulopathy, Fibroscan

INTRODUCTION

Hepatitis C almost always result in chronic liver disease i.e., lasting more than six months and can be called 'silent killer' as progression is usually gradual, sometimes very less alarming until fibrosis gets progressed to advanced stages.[1-3] According to a research conducted by U.S Department of Health and Human Services (HHS), 51% of persons living with hepatitis C infection do not know they have the virus. Death from hepatitis C is usually due to liver cirrhosis and hepatocellular carcinoma.[1] According to the report of 2015, 167000 deaths occurred from liver cancer while 326000 from cirrhosis in hepatitis C patients. More than 700000 people die from hepatitis C related diseases every year and prevalence of hepatitis is found to be higher in developing countries.[4-5]

HCV can cause both acute and chronic hepatitis.[2] It spreads by blood to blood contact involving intravenous drug abuse, blood transfusions, needles stick injuries or vertical transmission.[1] Its envelope proteins often vary their antigenic structure and our immune system can't keep up rendering the vaccine obsolete immediately. Hep C causes inflammation of liver which leads to jaundice, right upper quadrant pain and hepatomegaly and 60-80% patients become chronic.[4, 6-7] Lymphocytes infiltrate the portal tract and the chronic inflammation and infection lead to hepatocytes death. Liver cells become irritated, which leads to fibrosis and cirrhosis or alternatively hepatocytes go into frenzy and reproducing cells become malignant leading to hepatocellular carcinoma. Hep C infection leads to development of cryoglobulins or serum proteins containing IgM that precipitate and cool our temperature.[2,8]

Globally 71 million people are estimated to be chronically infected with Hep C. A significant number of those will develop cirrhosis or hepatocellular carcinoma. Pakistan has the world's second highest prevalence of the hepatitis

Table 1: Descriptive statistics

Fibrosis Stage		Platelet Count	ALT	AST	Alkaline Phosphatase	Bilirubin	Albumin	PT	APTT
F0-F1	Mean	288611.333	49.800	47.882	322.559		2.7525	14.864	35.103
	N	144	140	136	136	144	144	144	144
	Std. Deviation	80861.9997	33.4163	31.6928	141.8181		1.91158	.9115	3.0944
F2	Mean	161200.000	79.000	59.600	337.667		1.6600	14.840	35.400
	N	20	20	20	12	20	20	20	20
	Std. Deviation	56588.6356	44.6224	15.9552	121.5695		1.76826	1.1975	2.0105
F3	Mean	257428.571	74.714	67.286	252.500		2.3543	14.871	34.571
	N	28	28	28	24	28	28	28	28
	Std. Deviation	82391.7600	42.5300	47.6895	65.4583		1.77103	.9884	.7418
F4	Mean	161176.471	71.786	76.571	330.571		2.5982	15.429	35.765
	N	68	56	56	56	68	68	68	68
	Std. Deviation	51850.8024	46.3711	52.3759	143.3519		1.66260	1.0970	1.2231
Total	Mean	242123.200	60.098	57.817	317.947		2.5852	15.011	35.242
	N	260	244	240	228	260	260	260	260
	Std. Deviation	93279.7019	40.3571	40.3132	136.4401		1.83750	1.0195	2.4821

ALT=Alanine Aminotransferase, AST=Aspartate Aminotransferase, PT=Prothrombin Time, APTT=Activated Partial Prothrombin Time

C, second only to Egypt.[9] Coinciding high prevalence in parts of world where many people don't get effective diagnosis and prognosis due to requirement of expensive techniques, has made the fight with hepatitis tough.[10] In Pakistan, more than 60% of the population lives in rural areas. They do not have access to quality health facilities. Another huge problem is lack of awareness to get themselves screened for such infections periodically. Also, they can't bear cost of expensive tests like PCR, ELISA and Fibroscan which is also big hurdle in curbing prevalent hepatitis in developing countries.[11]

As Direct acting antivirals (DAAs) have made it possible to achieve 95 % cure rate. So the major hurdle today in our way to achieve HCV free world is the timely diagnosis of the infection and prognosis of liver fibrosis. This researches was carried out to assess how effective are serum biomarkers such as AST and ALT in predicting different stages of fibrosis.[1]

Almost all patients of liver impairment get their clotting profile done which is quit readily available test. As liver forms various pro- and anticoagulant factors as well as pro- and antifibrinolytic components, so in chronic hepatitis C infection and cirrhosis coagulopathy make it more fatal. Decreased concentration of all those clotting factors and essential proteins causing abnormally prolonged PT and APTT. The clotting factors involved in inactivated partial thromboplastin time (APTT) are I, II, V, VIII, IX, X, XI and XII. APTT is the more sensitive version of PTT, however, in APTT, an added activator that shortens clotting time making the reference range narrower. Severe infections and some cases of cancer can decrease PT, APTT. This prolonged PT and APTT is a one of the serious complication of chronic hepatitis C infection and cirrhosis.[12]

METHODS AND MATERIALS

This retrospective cross sectional study was conducted at Hepatitis Clinic, Lahore General Hospital, Lahore. HCV positive patients were identified using PCR and viral genotype was noted. Later, informed consent was obtained from patients who were willing to be involved in research. The analytical study was carried out from February 14, 2018 to January 14, 2019. It was made sure that patients having bleeding disorders or having any indication of disrupted coagulation system hemostasis like use of anticoagulation drugs were not included in our study. Also patients who had received an immunosuppressive therapy or had clinically diagnosed HBV or HIV or any type of liver cancer were not included in the study.

Fresh Blood samples were collected and PT, APTT tests were performed at Pathology Lab , Lahore General Hospital ,also CBC report was obtained to rule out any blood disorders and other liver function tests ALT,AST and Alkaline phosphatase, Albumin , Bilirubin were performed. The study was approved by Institutional Ethical Review Board (IERB), LGH.

Statistical analysis

The data was analyzed using statistical package SPSS windows version 22. A p value of less than 0.05 was considered statistically significant. To determine the significant association between continuous variables

Table 2: The Independent sample T- test results for stage F0-F1 & F2

	PT		APTT		p-value
Fibrosis Stage ⟶	F2	F0-F1	F2	F0-F1	
N	20	144	20	144	>0.05
Mean	14.840	14.864	35.400	35.103	
Std. Deviation	1.1975	.9115	2.0105	3.0944	
Std. Error Mean	.2678	.0760	.4496	.2579	

and liver fibrosis stages, Spearman's rank correlation was used. The One Way ANOVA and student t-test was used to compare arithmetic means and parameters. The univariate analysis was done for PT and APTT.

RESULTS

Frequencies

Total patients taken were 260, out of which 108 (41.5%) were male and 152 (58.5%) were female. Fibrosis stage demographics were F0-F1 =144 (55.4%), F2=20 (7.7%), F3=28 (10.8%), F4=68 (26.2%).

Descriptive statistics

The minimum and maximum values of PT and APTT were 13.7 & 17.5 and 26.0 & 46.2 respectively. The mean values and standard deviation values of patient age, baseline viral load, fibroscan score, Hb were 41.83 ± 12.95 ,984797.38 ± 2377269.10,11.42 ± 9.65 and 12.92 ± 3.62 respectively. The mean values and standard deviation of platelet count, ALT, AST, Alkaline Phosphatase, Albumin, PT, APTT are shown in **(table 1)** stage wise.

ANOVA

Analysis of variances (ANOVA) for PT and APTT showed a statistically significant relationship ($p<0.05$) with fibroscan score-determined fibrosis stage.

Independent samples T-test

The independent sample t-test results for stage F0-F1 and F2 ,PT and APTT variables showed non-significant relationship of PT and APTT with fibroscan score-determined fibrosis stage with p=0.932 and p=0.677) respectively as shown in **(table 2)**. The independent t-test results for stage F3 and F4 and PT and APTT variables showed a statistically significant relationship of PT and APTT with fibroscan score-determined fibrosis stage with p=0.022 and p=0.01 (both <0.05) respectively, as shown in **(table 3)**.

Univariate analysis of Variance

The relationship of PT and APTT with fibro scan score in univariate analysis was found to be statistically significant (p values <0.05) with R squared values of 0.886, 0.886 respectively.

DISCUSSION

Liver fibrosis as result of hepatitis C keeps on progressing gradually over the year, even decades with patients being unalarmed especially witnessed in developing countries due to poor prognosis of liver fibrosis and subsequently less accurate treatment approach in overburdened healthcare system as prevalence of hepatitis is coincidently higher in developing countries.[1-3] Several Non-invasive tests (NITs) particularly transient elastography i.e., fibroscan and combination of biomarkers are replacing invasive liver biopsy which has almost become obsolete owing to both healthcare burden and inconveniences and repercussions for patient, can have increased mortal mortality rate, also inter-observer variability and sampling error up to 30% included in drawbacks.[3,13-14] EASL recommendation also approve the preferential use of NITs over invasive biopsy.[3] Fibroscan inducted recently has been most preferred choice for prognosis of liver fibrosis if available and affordable to patient, it quite convenient and reliable but expensive and most importantly its availability is problematic in developing countries where burden is also higher in curbing prevalent hepatitis.[15]

Hepatitis is more prevalent in females than males and we got this consistent result in our study. Fibrosis stages F0-F1 and F4 stages were most commonly encountered with percentages 55.4% and 26.2% respectively.[16] There was significant relationship of progressed liver disease and age of patient as consistent with results of all other studies. Previously many studies made use of the combination of biomarkers in developing fibrosis serum indices, like ALT / AST ratio (AAR) fibro test (FT), fibrosis index (FI), AST platelet ratio (APRI) and FIB-4, all proclaimed to be predicting the prognosis with significant sensitivity and specificity. However final stage cirrhosis and mild fibrosis cannot be determined accurately by applying just one serum index, also all the readily available indices have some limitations like inability to differentiate all fibrosis stages individually and some have been developed primarily for co-infected patients.[17]

Blood markers have been used especially to predict cirrhosis and advanced stages of fibrosis and have been integral part of comprehensive assessment of patients presenting with liver impairment. Clotting Profile i-e PT, APTT are still in used as prognostic factors among LFTs.[18] There has been no study published so far comparing diagnostic performance of fibroscan and coagulation parameters. According to a study published PT, APTT was found deranged in most of chronic liver disease patients, APTT was prolonged in 71% and PT was raised in 88% in cases of chronic liver disease.[19] Coherently, Our results also indicate significant prolongation of PT, APTT in chronic cases. The minimum and maximum values of PT

Table 3: The Independent sample T- test results for stage F3 and F4

	PT		APTT		p-value
Fibrosis Stage ⟶	F4	F3	F4	F3	
N	68	28	68	28	>0.05
Mean	15.429	14.871	35.765	34.571	
Std. Deviation	1.0970	.9884	1.2231	.7418	
Std. Error Mean	.1330	.1868	.1483	.1402	

and APTT were 13.7 & 17.5 and 26.0 & 46.2 respectively in our study.

According to our results, for stage F0-F1 and F2 ,PT and APTT variables showed non-significant relationship of PT and APTT with fibroscan score-determined fibrosis stage with p=0.932 and p=0.677) respectively. for stage F3 and F4, PT and APTT variables showed a statistically significant relationship of PT and APTT with fibroscan score-determined fibrosis stage with p=0.022 and p=0.01 respectively which means that PT, APTT, simplest to perform among other lab NITs, can be used as prognostic markers efficiently at stages of advanced fibrosis and cirrhosis while initial stages of fibrosis don't affect PT, APTT significantly because patients with chronic liver disease fail to synthesize pro-and anticoagulant factors as well as pro-and antifibrinolytic components made by normal liver.

Limitation for implication of our study in individual cases can be patient factors like hypocalcemia, Vitamin K deficiency, endothelial dysfunction/damage, hypertension, infection and renal failure that may disrupt coagulation system hemostasis in cirrhotic patients and trigger factors like use of certain drugs inhibiting coagulation giving false positive results.[19]

CONCLUSION

The study implicates that, using PT, APTT lab tests, it can be predicted whether the patient having chronic course of hepatitis C, has progressed to F3, cirrhosis (F4) or not without reliance on Fibroscan to confirm diagnosis of cirrhosis. PT,APTT are severely affected and prolonged in F3 and F4 but F0-F1 and F2 stages does not affect them significantly because various pro- and anticoagulant factors as well as pro- and antifibrinolytic components made by liver are affected greatly in chronic hepatitis C infection and cirrhosis leading to coagulopathy.

REFERENCES

1. Vento S1, Cainelli F. Does hepatitis C virus cause severe liver disease only in people who drink alcohol? Lancet Infect Dis. 2002; 2(5):303-9.
2. Vegnente A, Larcher VF, Mowat AP, Portmann B, Williams R.Duration of chronic active hepatitis and the development of cirrhosis. Arch Dis Child. 1984;59(4):34.
3. EASL Clinical Practice Guidelines: Management of hepatitis C virus infection. Journal of Hepatology. 55. 10.1016/j.jhep.2013.11.003.
4. World Health Organization. Pakistan tackles high rates of hepatitis from many angles. 11 July 2017.
5. Hussain A, Noor HT, Nabi U (2018) Evaluation of Role of Increased Perceived Stress Score and Body Mass Index in Causing Secondary Systemic Hypertension in Patients of Hepatitis C. J Cardiovasc Dis Diagn 6: 343.
6. Hussain A, Zubair, Tabassum MS. Can We Replace Transient Elastography i.e. Fibro scan with cheap biomarkers? A cross sectional study at Lahore General Hospital, Lahore. Indo Am. J. P. Sci, 2019; 06(05). 9518-9531.
7. Hussain A, Khalid I, Fatima R, Gull MA. Relationship of Derangements in Various Blood Indices and Coagulopathy with Liver Stiffness Index (LSI) In Various Stages of Fibrosis In Hepatitis C., Indo Am. J. P. Sci, 2019; 06(05). 9533-9541.
8. Tsochatzis EA, Bosch J, Burroughs AK.Liver cirrhosis. Lancet 2014;383:1749-61.
9. Aaron G Lim,1 Huma Qureshi,2 Hassan Mahmood,2,3 Saeed Hamid,4 Charlotte F Davies,1 Adam Trickey, et al. Curbing the hepatitis C virus epidemic in Pakistan: the impact of scaling up treatment and prevention for achieving elimination. Int J Epidemiol. 2018 Apr; 47(2): 550–560.
10. Siddiqui SA, Ahmed M, Ghani MH, Memon MA, Mustafa G, Ghori MA. Coagulation abnormalities in patients with chronic liver disease in Pakistan. . J Pak Med Assoc. 2011 Apr; 61(4). 363-367
11. Keltch B, Lin Y, Bayrak C. Comparison of AI techniques for prediction of liver fibrosis in hepatitis patients. J Med Syst. 2014;38(8):60.
12. Northup PG, Caldwell SH. Coagulation in liver disease: a guide for the clinician. Clin Gastroenterol Hepatol. 2013;11:1064–1074.
13. Thampanitchawong P, Piratvisuth T. Liver biopsy: complications and risk factors. World Journal of Gastroenterology 1999; 5(4):301-304.
14. Sanai, F. M., Keeffe, E. B. (2010). Liver biopsy for histological assessment: The case against. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association, 16(2), 124-132.
15. Chahal HS, Marseille EA, Tice JA, et al. Cost-effectiveness of Early Treatment of Hepatitis C Virus Genotype 1 by Stage of Liver Fibrosis in a US Treatment-Naive Population. JAMA Intern Med. 2016;176(1):65–73.

16. Bakr I, Rekacewicz C, El Hosseiny M, et al. Higher clearance of hepatitis C virus infection in females compared with males. Gut. 2006;55(8):1183-7.
17. Ahmad W1, Ijaz B, Javed FT, Gull S, Kausar H, Sarwar MT, et al. A comparison of four fibrosis indexes in chronic HCV: Development of new fibrosis-cirrhosis index (FCI). BMC Gastroenterology 2011. 4 BMC Gastroenterol. 2011 Apr 21;11:44.
18. Thachil J: Relevance of clotting tests in liver disease. Postgraduate Medical Journal 2008;84:177-181.].
19. Capoor MN1, Stonemetz JL2, Baird JC3, Ahmed FS3, Awan A4, Birkenmaier C5, et.al. Prothrombin Time and Activated Partial Thromboplastin Time Testing: A Comparative Effectiveness Study in a Million-Patient Sample. PLoS One. 2015 Aug 11;10(8):e0133317

ORIGINAL ARTICLE

RELATIONSHIP OF MICROCYTIC HYPOCHROMIC AND NORMOCYTIC HYPOCHROMIC ANEMIA WITH LIVER STIFFNESS INDEX IN HEPATITIS C PATIENTS

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ABSTRACT

Background:
Chronic hepatitis disease is frequently associated with anemias among the complications. We tried to find out co relation between Liver stiffness index (LSI) and probability to have microcytic and normocytic hypochromic anemias in hepatitis C patients.

Methods:
It is prospective study, conducted at hepatitis clinic, LGH. Total 177 HCV patients having with PCR done were taken, LFTs were performed, and patients having any indication of blood pathologies were excluded from our study. Spearman's rank correlation was used to determine significant correlation with continuous variables and liver fibrosis stages.

Results:
Chi square test and T test for different variables i-e Baseline viral load, Hb, Hematocrit, MCV, MCH, MCHC showed statistically significant relationship ($p<0.05$) with fibroscan score i-e increased in later stages of fibrosis.

Conclusion and Implications:
This study signifies and elaborates different types of anemias such as microcytic hypochromic and normocytic hypochromic anemia with different stages of fibrosis. We recommend continuous monitoring of hemoglobin, transferrin saturation and serum ferritin levels in chronic hepatitis C. Iron supplements and Recombinant Human Erythropoietin should be administered if any of these anemias is found.

Key Words:
Fibroscan, Chronic hepatitis C, Anemias, Liver fibrosis

INTRODUCTION

Hepatitis C virus (HCV) is an infectious pathogen causing enormous damage to the liver.[1] It is the common cause of chronic liver disease leads toward liver hepatocellular carcinoma and cirrhosis.[2] It is affecting 180 million individuals of the world and 10 million people in Pakistan.[3] About 3–4 million individuals are affected by HCV annually.[4] Hepatitis C virus (HCV) represents a considerable morbidity and cost burden, contributing to approximately 400,000 deaths annually worldwide and estimated yearly costs of at least US$6.5 billion in the United States.[5-6] At present, HCV treatment has numerous side effects like anemia, neutropenia, leukopenia, and thrombocytopenia.[7-8] Preliminary data suggest that the infection itself can also induce autoimmune hemolytic anemia, leukopenia, and thrombocytopenia.[9] Ribavirin (RBV) therapy causes accumulation of drugs in RBCs results in hemolytic anemia which leads towards dose adjustment and discontinuation of therapy. Ribavirin-induced hemolysis is passive and non-inflammatory/non-immune-mediated. Ribavirin-induced hemolysis is also dose-related and sustained. The reduction in hemoglobin levels appears to correlate directly with the degree of hemolysis and inversely with the erythropoetic ability of the bone marrow. Hemolysis and reduced hemoglobin can lead to major side effects; however, treatment-related anemia, particularly during the first 4-8 weeks, correlates well with the efficacy (SVR rate) of the combination therapy.[10]

Acute hepatitis is asymptomatic in 70-80 percent of individuals.11In chronic hepatitis C, the viral RNA persists in blood at least 6 month after acute infection and chronic hepatitis is develop in 75-80% of patients by 6 months as they do not clear the virus. Factors including the age at time of infection, gender, ethnicity and development of jaundice during acute infection are determinant of rate chronic HCV.[11] Antibody detection assays do not provide a means to differentiate between current, active infection; chronic infection; and past, resolved infection. Polymerase chain reaction (PCR) amplifies the viral nucleic acids so it has been shown to be an effective means for the direct detection of HCV.[12]

Table 1: Descriptive statistics

Variables	N	Minimum	Maximum	Mean	Std. Deviation
Patient Age	177	22	71	39.95	12.478
Baseline Viral Load	177	221	13959308	464008.76	1806011.644
FibroScan Score	177	2.70	32.00	10.2627	7.46133
Platelet Count	177	150	579000	264595.76	129837.719
MCV	177	18.3	102.0	78.493	14.9668
MCH	177	.3	36.1	25.307	6.2986
MCHC	177	22.8	40.7	30.983	3.3838
Hb	177	7.0	13.0	10.888	1.4169
HCT	177	21.60	45.00	35.4049	5.01060
Valid N (List Wise)	177				

We tried to evaluate whether increased prevalence of microcytic hypochromic and normocytic hypochromic anemia is associated with Increased liver stiffness index (LSI) in hepatitis C patients causing them fatigue and weakness.

METHODS AND MATERIALS

This prospective study was conducted at Hepatitis Clinic, Lahore General Hospital (LGH), Lahore. HCV positive patients were identified using PCR and viral genotype was noted. Later, informed consent was obtained from patients who were willing to be involved in research. The analytical study was carried out from February 1, 2018 to January 11, 2019. It was made sure that patients having bleeding disorders or having any indication of disrupted coagulation system hemostasis like use of anticoagulation drugs were not included in our study. Also patients who had received an immunosuppressive therapy or had clinically diagnosed HBV or HIV or any type of liver cancer were not included in the study.

Fresh Blood samples were collected and liver function tests AST, ALT , Albumin, Alkaline phosphatase and Bilirubin were performed at Pathology Lab , Lahore General Hospital, also CBC report was obtained to rule out any blood disorders and other. The study was approved by Institutional Ethical Review Board (IERB), LGH.

Statistical analysis

The data was analyzed using statistical package SPSS windows version 22. A p value of less than 0.05 was considered statistically significant. To determine the significant association between continuous variables and liver fibrosis stages, Spearman's rank correlation was used. The Chi Square test was used to compare categorical data and student t-test was used to compare arithmetic means and parameters.

RESULTS

Frequencies

Total patients taken were 177 ; 47 (26.6%) were males , females were 130 (73.4 %).

Descriptives Statistics

Mean values of Patient Age, Baseline Viral Load, fibroscan score Platelet Count, MCV, MCH, MCHC, Hb and Hct were 39.95, 464008.76, 10.2627, 264595.76, 78.493, 25.307, 30.983, 10.888, 35.4049 respectively **(table 1)**.

Chi square test

Chi square results for different variables i.e. Baseline viral load, Hb, Hematocrit, MCV, MCH and MCHC with Fibro scan score in microcytic hypochromic anemia and normocytic hypochromic anemia are statistically significant with $p < 0.05$ respectively.

T-test

The independent sample T-test results showed a statistically significant relationship of fibroscan score with different variables i-e Patient Age, Baseline Viral Load, Platelet Count, MCV, MCH, MCHC, Hb and Hct in different fibrosis stages in microcytic hypochromic anemia and normocytic hypochromic anemia with p=0.41, p=0.16, p=0.19, p=0.00, p=0.00, p=0.00, p=0.01 and p=0.00 respectively

DISCUSSION

In Liver fibrosis as result of chronic hepatitis C keeps on progressing gradually over the year, even decades with patients being unalarmed especially witnessed in developing countries due to poor prognosis of liver fibrosis and subsequently less accurate treatment approach in overburdened healthcare system as prevalence of hepatitis is coincidently higher in developing countries.[1,3,5] Cirrhosis doesn't develop simultaneously but it takes a mean infection time of approximately 30 years, but it may occur in different ages in different age ranges i.e., 10-50 years.[3-4] Fibrosis in connective tissue followed by its extension in hepatic tissue in hepatitis C infection is a evidence of cirrhosis.[13] Liver fibrosis determines the basis upon which treatment with interferon therapy depends so comparison with fibroscan score has importance. Previously many studies tried to determine the ties

between aminotransferases level, hyaluronic acid levels, number of platelets, collagen levels and Baseline viral load with fibrosis but to no avail, as results were uncertain. Prognosis can't be determined by one structure only. Our study has implications in follow up.

Anemias are among common complications of chronic liver disease related to therapy. The results of Independent case studies have shown that patients with chronic HCV infection can develop autoimmune hemolytic anemia in the absence of treatment with Interferon (IFN-a).[9-10,14-15] During the last decade, the discovery of the anti-inflammatory properties of heme oxygenase-1 (HMOX1) and the presence of oxidative stress in patients with chronic HCV has caused many researchers to evaluate the use of HMOX1 as a therapeutic option. Ribavirin-induced hemolysis provides sufficient heme that increases HMOX1 in Kupffer cells and decreases inflammation; increases HMOX1 in hepatocytes, which decreases the oxidative damage and apoptosis caused by HCV and which slightly decreases HCV replication.[16]

In Pakistan, 3a was the most prevalent genotype and our study also depicted same results.[17] There were large number of patients with F0-F1 i-e none or initial fibrosis stage while F2 and F3 stages of fibrosis, and cirrhosis (F4) were a remarkably associated with older ones; lack of awareness and low socioeconomic status. Hepatitis is more prevalent in females than males and we got this consistent result in our study. Fibrosis stages F0-F1 and F4 stages were most commonly encountered with percentages 55.4% and 26.2% respectively. There was significant relationship of progressed liver disease and age of patient as consistent with results of all other studies Several Non-invasive tests (NITs) particularly transient elastography i.e., fibroscan and combination of biomarkers are replacing invasive liver biopsy which has almost become obsolete owing to both healthcare burden, inability to follow up and inconveniences and repercussions for patient ,can even be mortal [1.6% mortality rate observed in a study, also inter-observer variability and sampling error up to 30%(11) included in drawbacks.[5-6] EASL recommendation also approve the preferential use of NITs over invasive biopsy.[5] Fibroscan inducted recently has been most preferred choice nowadays for prognosis of liver fibrosis if available and affordable to patient, it is quite convenient and reliable but expensive and most importantly its availability is problematic in developing countries where burden is also higher in curbing prevalent hepatitis.[6]

Blood markers have been used especially to predict cirrhosis and advanced stages of fibrosis and have been integral part of comprehensive assessment of patients presenting with liver impairment, used as prognostic factors among LFTs.[12] There has been no study published so far comparing diagnostic performance of fibroscan and LFTs. According to a study published PT, APTT was found deranged in most of chronic liver disease patients.[17]

Final stage cirrhosis and mild fibrosis cannot be determined accurately by applying just one serum index, also all the readily available indices have some limitations like inability to differentiate all fibrosis stages individually and some have been developed primarily for co-infected patients.[18] Stage wise relationship can be used as prognostic markers efficiently at stages of advanced fibrosis and cirrhosis while initial stages of fibrosis don't affect significantly because patients with chronic liver disease fail to synthesize components made by normal liver.

We recommend continuous monitoring of hemoglobin, transferrin saturation and serum ferritin levels in chronic hepatitis C. Iron supplements and Recombinant Human Erythropoietin should be administered if any of these anemias is found.

Limitation for implication of our study in individual cases can be patient factors that are responsible for anemias and other blood disorders, cause of which not related to liver disease. This can alter results so we have taken care of it in methods as well.

CONCLUSION

The probability of both microcytic and normocytic hypochromic anemias increases in F3 and F4 stages and prevention therapy is recommended. This study signifies and elaborates different types of anemias such as microcytic hypochromic and normocytic hypochromic anemia with different stages of fibrosis.

We recommend continuous monitoring of hemoglobin, transferrin saturation and serum ferritin levels in chronic hepatitis C. Iron supplements and Recombinant Human Erythropoietin should be administered if any of these anemias is found.

REFERENCES

1. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Maqder LS, et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet, 85 (2000), pp. 5887-5891
2. Kanda T, Yokosuka O, Omata M. Hepatitis C virus and hepatocellular carcinoma. Biology, 2 (2013)
3. Raja NK, Janjua KA et al. Epidemiology of hepatitis C virus infection in Pakistan. J Microbiol Immunol Infect, 41 (2008), pp. 4-8
4. Berenguer M, Lopez-Labrador FX, Wright TL. Hepatitis C virus transplantation J Hepatol, 35 (2001), pp. 666-678
5. Hill AM, Nath S, Simmons B. The road to elimination of hepatitis C: analysis of cures versus new infections in 91 countries. J Virus Erad 2017; 3: 117– 123.
6. Razavi H, Elkhoury AC, Elbasha E et al. Chronic hepatitis C virus (HCV) disease burden and cost in the United States. Hepatology 2013; 57: 2164– 2170.
7. Dusheiko G, Main J, Thomas H, et al. Ribavirin treatment for patients with chronic hepatitis C: results of a placebo-controlled study. J Hepatol 1996; 25:591–8.
8. Maddrey WC. Safety of combination interferon alfa-2b/ribavirin therapy in chronic hepatitis C–relapsed and treatment-naï¨ve patients. Semin Liver Dis 1999;

19 (Suppl 1):67–75.
9. Streiff MB, Mehta S, Thomas DL. Peripheral blood count abnormalities among patients with hepatitis C in the United States. Hepatology 2002; 35:947–52.
10. Sulkowski MS, Shiffman ML, Afdhal NH, Reddy KR, McCone J, Lee WM, Herrine SK, Harrison SA, Poordad FF, Koury K, et al. Hepatitis C virus treatment-related anemia is associated with higher sustained virologic response rate. Gastroenterology. 2010; 139:1602–1611, 1611.e1.
11. Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection Int J Med Sci, 3 (2006), pp. 47-52
12. Okamoto, H., S. Okada, Y. Sugiyama, T. Tanaka, Y. Sugai, Y. Akahane, A. Machida, S. Mishiro, H. Yoshizawa, Y. Miyakawa, and M. Mayumi. 1990. Detection of hepatitis C virus RNA by a two-staged polymerase chain reaction with two pairs of primers deduced from the 5'-noncoding region. Jpn. J. Exp. Med.
13. Afridi SQ, Zahid MN, Shabbir MZ, Hussain Z, Mukhtar N, Tipu MY, et al. Prevalence of HCV genotypes in district Mardan. Virol J, 10 (2013), pp. 10-90
14. Manns MP, McHutchison JG, Gordon SC, et al. Peg interferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001; 358: 958–65.
15. Moccia F, Tognoni E, Boccaccio P. Autoimmune hemolytic anemia in chronic hepatitis C virus infection: an unusual extrahepatic autoimmune manifestation. Ann Ital Med Int 2001; 16:256–9.
16. Soota K, Maliakkal B. Ribavirin induced hemolysis: A novel mechanism of action against chronic hepatitis C virus infection. World J Gastroenterol. 2014 Nov 21; 20(43): 16184–16190.
17. Hill AM, Nath S, Simmons B. The road to elimination of hepatitis C: analysis of cures versus new infections in 91 countries. J Virus Erad 2017; 3: 117– 123.
18. Chamot, E., B. Hirschel, J. Wintsch, C.-F. Robert, V. Gabriel, J.-J. Deglon, S. Yerly, and L. Perrin. 1990. Loss of antibody against hepatitis C virus in HIV-seropositive intravenous drug users. AIDS 4:1275-1277.

ORIGINAL ARTICLE

VALIDATION OF REPLACEMENT OF TRANSIENT ELASTOGRAPHY (FIBRO SCAN) WITH COMBINATION OF NON-INVASIVE, CHEAP AND READILY AVAILABLE BIOMARKERS, A PROSPECTIVE CROSS-SECTIONAL STUDY

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ABSTRACT

Introduction:
Hepatitis C is a leading cause of liver fibrosis, cirrhosis and cirrhosis associated complications. In this study, we compared readily available non-invasive fibrosis indices and biomarkers for the fibrosis staging and predicting its progression in Pakistani population.

Methods:
The prospective cross sectional study was conducted in medicine wards and hepatitis clinic of LGH/AMC, Lahore from February 12, 2018 to January 11, 2019. We studied 1898 HCV infected patients which were got CBC, LFTs, ELISA and PCR done to perfectly diagnose ongoing hepatitis C infection and then, fibro scan were performed for staging of fibrosis. In order to differentiate HCV fibrosis progression, we compared the prognostic effectiveness of fibro scan score (Liver Stiffness Index, LSI) and fibrosis stages assessed by fibro scan score with readily available serum indexes ; AST to ALT Ratio (AAR), AST to Platelet Index (APRI), Fibrosis Index (FI), FIB-4, Age to Platelet Index (API), Pohl score, and Fibrosis Cirrhosis Index (FCI).

Results:
Readily available serum indexes AST to ALT Ratio (AAR), AST to Platelet Index (APRI), Fibrosis Index (FI), FIB-4, Age to Platelet Index (API), Pohl score and Fibrosis Cirrhosis Index (FCI) were able to stage liver fibrosis with correlation coefficient indexes 0.848,0.711, 0.003, 0.618, 0.741, 0.529, 0.360 and 0.477 respectively.

Conclusion and Implications:
Readily available and cheap serum indexes AST to ALT Ratio (AAR), AST to Platelet Index (APRI), Fibrosis Index (FI), FIB-4, Age to Platelet Index (API), Pohl score and Fibrosis Cirrhosis Index (FCI) predicted fibrosis stages in HCV infected patients in co-relation to the costly and less accessible Fibro scan with considerable accuracy.

Key Words:
Fibroscan, Chronic hepatitis C, Non-invasive liver marker, Liver fibrosis, Accuracy, Equivalence

INTRODUCTION

Hepatitis C is the most prevalent cause of as a major cause of liver related disorders in this world. Prevalence of HCV infected people reaches up to 3 people out of one hundred world's population and total infected were10 million.[1-3] Most common genotypes in Pakistan are 1a, 4a, 3a and 3b.[4] Liability to develop hepatocellular carcinoma(HCC) in HCV infected people reaches up to 1-4% following onset of cirrhosis, with cirrhosis occurring in 10-15 years of age range.[5] Liver biopsy has been gold standard for evaluation of fibrosis stages. Yet it is an invasive procedure, costly accompanied by serious side effects as soreness, pain and severe complications that can lead to death.[1,3,5] Various researches narrated the host factors contemplating the fibrosis development that can ultimately lead to HCC.[6-7] Their usage is compatible as NITs to overcome the drawbacks of invasive procedure. Hepatitis C causes hepatic insult which linked to abnormally high levels of A L T and AST in blood 6 months. Stage of liver fibrosis determines the basis upon which treatment with interferon therapy depend.[8] Previously many studies tried to find out the authentic, non-afflicting biomarkers and tried to determine the ties between aminotransferases level, hyaluronic acid levels, number of platelets, collagen levels and HCV viral titer with fibrosis but to no avail, as results were uncertain. Since then different thresholds of several scoring systems like ALT / AST ratio (AAR) fibro test (FT), fibrosis index (FI), AST platelet ratio (APRI) and FIB-4 have been proclaimed to anticipate the existence and absence of fibrosis or cirrhosis in HCV infected patients. Previously many studies tried to find out the authentic, non-afflicting biomarkers and tried to determine the ties

Table 1: Gender distribution of 1898 patients

Gender	Frequency (N)	Percent (%)	Valid Percentage (%)	Cumulative Percent (%)
Female	1224	64.5	64.5	64.5
Male	674	35.5	35.5	100
Total	1898	100	100	

between aminotransferases level, hyaluronic acid levels, number of platelets, collagen levels and hepatitis viral load with fibrosis and results were not significant. Since then different thresholds of various assessment criteria like ALT / AST ratio (AAR) fibro test (FT), Fibrosis Index, AST Platelet Ratio and Fibrosis-4 have been proclaimed to anticipate the presence of fibrosis or cirrhosis in HCV patients. However final stage cirrhosis and mild fibrosis cannot be determined accurately by using only one system.[9-18] In following study, easily accessible biomarkers as ALT/AST, FT, Fibrosis Index and Fibrosis-4 were not only contrasted but also assessed for their diagnostic abilities to determine the authentic biomarkers for estimation of liver fibrosis.

Table 2: Marital status of patients

Marital Status	Frequency (N)	Percent (%)	Valid Percentage (%)	Cumulative Percent (%)
Married	1770	93.5	93.5	93.1
Unmarried	123	6.5	6.5	99.6
Total	1898	100	100	

METHODS

This study was held at Lahore General Hospital, Lahore. HCV positive patients were identified. Later ,we threw light on our study plan for clarification of patient's concepts about the whole process and informed consent from patients who were willing to involve in procedure .This was a prospective cross- sectional study. This study took place from February 12, 2018 - January 11, 2019.

Fibrosis staging were performed on the basis of fibroscan score which was carried out at Medicine Department, Ameer- ud-din Medical College, Lahore, in accordance with METAVIR assessment criterion [19]. Fibrosis have five degrees of fibrosis starting from F0= no fibrosis, F1=mild fibrosis having no septa, F2 =moderate fibrosis with a few septa, F3=intense fibrosis with numerous septa and no cirrhosis and F4=cirrhosis.

We used QI A amp Viral RNA Extraction Kit RNA was obtained from 140 micro litres serum samples. M mLV, reverse transcriptase and Invitrogen were used to replicate cDNA. Taq Polymerase (Fermentas USA) was used to carry out first round and nested PCRs and 2% agarose gel was used for analysis. Using Third Wave Technology of USA, HCV genotyping was done for 12 different genotypes of Hepatitis C virus.

For further biochemical evaluation, samples of serum collected from different subjects which were kept at -70°C. Different assessment tools like liver function tests (LFTs), bilirubin levels, heamoglobin value, albumin levels and PLT were calculated for every subject.

The patients were evaluated for AAR, APRI, FI, FIB-4, API, Pohl score and FCI. The following formulas were used to review the predicted scores with particular cut-off values.

- AST to ALT Ratio (AAR) = AST(IU/l)/ALT(IU/l),

AST to ALT Ratio (AAR) ≥ 1, cirrhosis is suspected.

- AST to Platelet index (APRI) = [{AST (IU/l)/ALT_ULN (IU/l)}×100]/platelet count(109/l).

If AST to Platelet index (APRI) <0.5, minimal or no fibrosis found; if AST to Platelet index (APRI) > 1.5, significant cirrhosis present.

- Fibrosis Index (FI) = 8.0-0.01×PLT(109/l)-serum albumin(g/dl)

If Fibrosis Index (FI); <2.1, minimal fibrosis; if ≥2.1, significant fibrosis, and if ≥ 3.3, significant cirrhosis.

- Fibrosis-4 (FIB-4) = [Age(Years)×AST(IU/l)]/[Platelet

Table 3: Genotype of patients

Genotype	Frequency (N)	Percent (%)	Valid Percentage (%)	Cumulative Percent (%)
3A	1235	65.1	65.1	65.1
1B	581	30.6	30.6	95.7
1A	82	4.3	4.3	100
Total	1898	100	100	

Table 4: Fibrosis stage among patients

Fibrosis Stage	Frequency (N)	Percent (%)	Valid Percentage (%)	Cumulative Percent (%)
F0-F1	1034	54.5	54.5	54.5
F2	112	5.9	5.9	60.4
F3	253	13.3	13.3	73.7
F4	499	26.3	26.3	100
Total	1898	100	100	

count(×109/l)×ALT(IU/l)1/2]

If Fibrosis-4 (FIB-4) <1.45, no / minimal fibrosis. If Fibrosis-4 (FIB-4)>3.25, significant cirrhosis.

- Fibrosis Cirrhosis Index (FCI) = (Alkaline Phosphatase × Serum Bilirubin/Serum Albumin × Platelet count)

If FCI < 0.131, significant fibrosis; If FCI>1.25, significant cirrhosis.

- Age to Platelet index (API) = Age/ Platelet index
- Pohl Score; AST : ALT : Platelet Count (10^9/L)

If Pohl Score is less than 1 and PLT > 150,000 then no / minimal fibrosis found.

Statistical analysis

SPSS windows version 22 was used to analyze the data. p value of less than 0.05 was considered statically significant. To signify the marked association between stages of liver fibrosis and continuous variables, Spearman's rank correlation was used. We used student t-test to relate arithmetic means and parameters while to relate categorical data we used chi square(X2). Various univariate and multivariate regression analysis was performed for multiple biomarkers. Receiver Operating Curves (ROC) and AUROC were performed to infer the diagnostic precision of the serum fibrosis indexes along with their cutoff points, sensitivities and specificities.

RESULTS

We studied data of 1898 patients with 1224 (64.5%) patients are female and 674 (35.5%) are male shown in **(table 1)**. According to data of marital status 1767(93.1%) patients are married while 123(6.5%) are unmarried **(table 2)**. Out of them 1235(65.1%) patients had genotype 3a, 581(30.6%) had 1b and 82(4.3%) had 1A genotype **(table 3)**.

Table 5: Descriptive statistics

Variables	N	Minimum	Maximum	Mean	Std. Deviation
Age of Patient	1893	14.0	100.0	41.550	12.8913
Baseline Viral Load	1898	119	107911144	1101257.94	6075925.912
Albumin	1898	0.70	15.00	3.6440	1.36541
ALT	1898	8.0	7000.0	78.195	171.6666
AST	1898	14.0	1085.0	74.195	64.9119
Bilirubin	1898	.20	24.00	1.1946	1.22437
Fibroscan Score	1898	2.60	76.00	13.3569	13.00018
Platelet Count	1898	17900.00	26800000.00	502315.3061	2299095.85128
AAR	1898	.05	9.94	1.0697	.54862
Platelet Count	1898	17.90	26800.00	502.3153	2299.09585
APRI	1898	.00	8.24	.8769	1.03031
FI	1898	-262.72	6.20	-.3871	22.94007
Fib-4	1898	.00	23.28	1.7149	1.88862
FCI	1898	.00	47.72	.7808	1.98782
Alkaline Phosphatase	1898	51.0	1154.0	301.946	137.8091
API	1898	.0	10.0	2.893	2.8627
Pohl Score	1898	.0	1.0	.663	.4729
Valid N (List Wise)	1893				

Table 6: T-test results of F0-F1 and F2

Test	Fibrosis Stage	N	Mean	Std. Deviation	Std. Error Mean	p value
AAR	F0-F1	1034	1.1177	.69027	.02147	<0.05
	F2	112	1.0000	.14077	.01330	
APRI	F0-F1	1034	.5390	.65438	.02035	<0.05
	F2	112	.7501	.61264	.05789	
FI	F0-F1	1034	-2.9709	30.81657	.95835	<0.05
	F2	112	1.4462	2.52292	.23839	
Fib-4	F0-F1	1034	1.1855	1.36602	.04248	<0.05
	F2	112	1.4378	.75666	.07150	
FCI	F0-F1	1034	.2714	.40388	.01256	<0.05
	F2	112	.3003	.22633	.02139	
API	F0-F1	1034	2.358	2.6433	.0822	<0.05
	F2	112	2.750	2.7097	.2560	
Pohl Score	F0-F1	1034	.640	.4802	.0149	<0.05
	F2	112	.821	.3847	.0364	

The determination of fibrosis stage among HCV infected patients depicts that among 1898 patients 1034 (54.5%) patients are in fibrosis stage F0-F1 stage, 112 (5.9%) patients are in F0-F1 stage, 253 (13.3%) patients in F3 and 499 (26.3%) patients are in F4 leading cirrhosis **(table 4)**.
The means and standard deviations of age of patients, Baseline Viral Load, Albumin, ALT, AST, Bilirubin, Fibroscan score, AAR, Platelet count, APRI, FI, Fib-4, FCI, Alkaline Phosphatase, API, Pohl Score and NFI were 41.5±12.8, 1101257.9±6075925.9, 3.6±1.3, 78.1±171.6, 74.1±64.9, 1.1±1.2, 13.3±13.0, 1.0±0.5, 502.3±2299.0, 0.8±1.0, 0.3±22.9, 1.7±1.8, 0.8±1.9, 301.9±137.8, 2.8±2.8, 0.6±0.4, 7498.4±14670.7 respectively **(table 5)**.
The Independent sample T-test results for stage F0-F1 & F2 for different variables i.e. AAR, APRI, FI, Fib-4, FCI, API and Pohl Score is given in the table below showing statistically significant relationship of all these variables with fibrosis stages of F0-F1 and F2 determined fibro scan

Table 7: T-test results of F3 & F4

Test	Fibrosis Stage	N	Mean	Std. Deviation	Std. Error Mean	p value
AAR	F3	253	.9515	.20530	.01291	<0.05
	F4	499	1.0459	.34483	.01544	
APRI	F3	253	.8005	.62448	.03926	<0.05
	F4	499	1.6443	1.42850	.06395	
FI	F3	253	2.3157	1.27981	.08046	<0.05
	F4	499	3.1850	1.16513	.05216	
Fib-4	F3	253	1.5611	1.11664	.07020	<0.05
	F4	499	2.9519	2.61027	.11685	
FCI	F3	253	.6640	.95608	.06011	<0.05
	F4	499	2.0036	3.48480	.15600	
API	F3	253	4.008	3.1155	.1959	<0.05
	F4	499	3.467	2.9351	.1314	
Pohl Score	F3	253	.834	.3728	.0234	<0.05
	F4	499	.587	.4928	.0221	

Table 8: Univariate analysis results

Variable	AAR	APRI	FI	Fib-4	FCI	API	Pohl Score
p value	.000	.000	.000	.000	.000	.000	.000
R squared value	.942	.986	.840	1.000	.999	.171	.004

score with p<0.05 **(table 6)**.

The student T- test results for stage F3 & F4 for different variables i.e. AAR, APRI, FI, Fib-4, FCI, API and Pohl Score is given in the table below showing statistically significant relationship of all these variables with fibrosis stages determined fibro scan score with p<0.05 **(table 7)**.

Univariate analysis for AAR, APRI, FI, Fib-4, FCI, API and Pohl score showed a statistically significant relationship with Pearson's correlation coefficients (R) values given below in the **(table 8)**.

Linear curve estimation analysis with Analysis of variances (ANOVA) for AAR, APRI, FI, Fib-4, FCI, API and Pohl score showed a statistically significant relationship with Spearman's correlation coefficients (R) values of .087 (p<0.05), .492 (p< .05), .091 (p<.05), .334 (p<.05), .568 (p<.05), .234 (p<.05) & .066 (p<.05) respectively in **(figure 1)**.

ROC Curve analysis for validation of serum ALT/AST ratio, AST platelet ratio APRI, Fibrosis 4, Fibrosis index , API, Pohl score and FCI were performed and sensitivity and

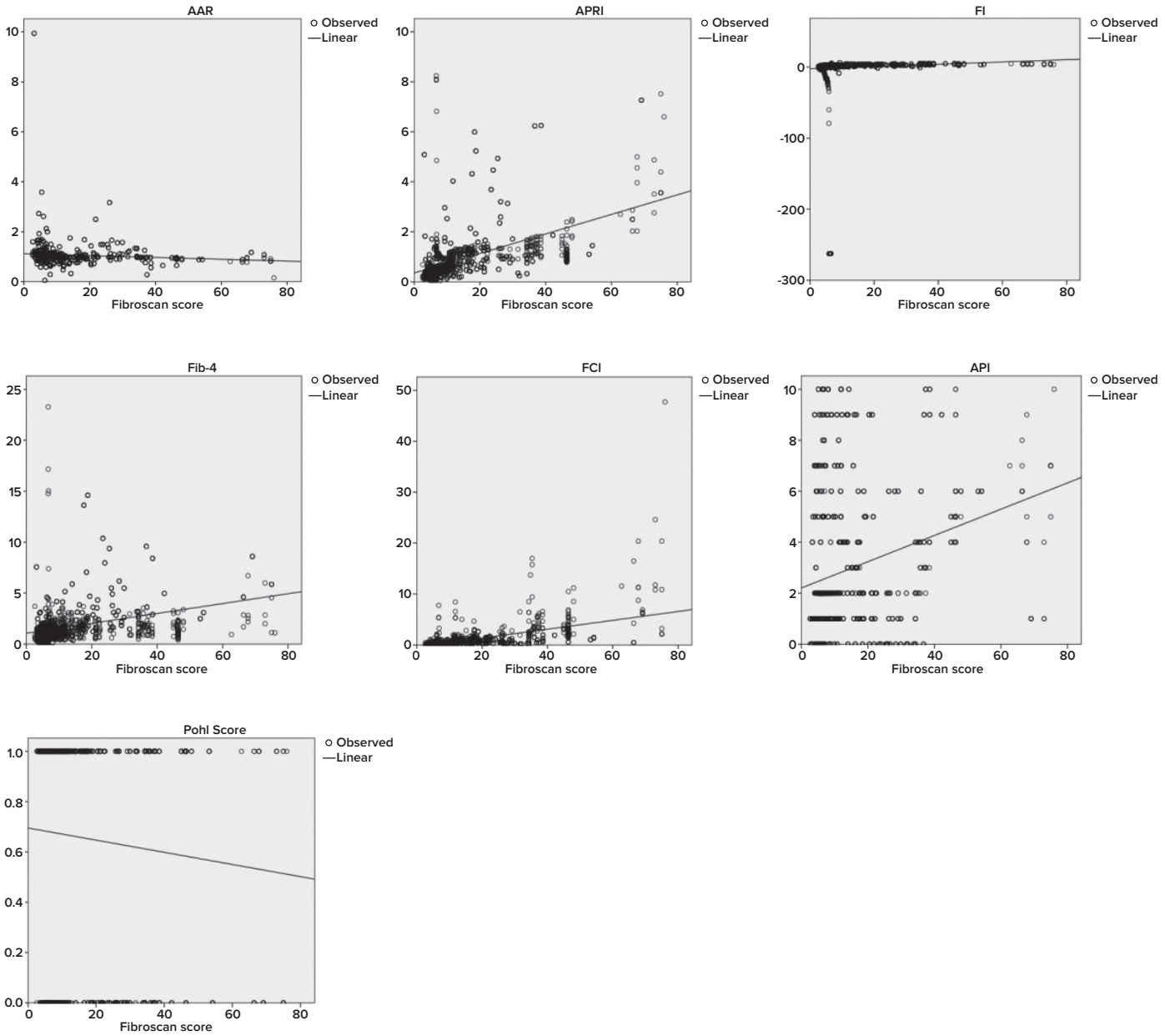

Figure 1: Linear curve analysis of variances (ANOVA) for AAR, APRI, FI, Fib-4, FCI, API and Pohl score

Table 9: ROC Curve analysis for validation of serum AAR, APRI, FIB-4, FI, API, Pohl score and FCI for F0-F3 and F4 in 1898 HCV infected patients

Stage	Cutoff Value	Spec %	Sens %	AUC
AAR				
F0-F3	< 1	41.9	62.5	0.377
F4	> 1	37.6	62.8	0.412
APRI				
F0-F3	< 0.5	68.0	56.2	0.54
F4	> 1.5	87.6	74.8	0.864
FIB-4				
F0-F3	< 1.45	65.4	51	0.521
F4	> 3.25	72.3	53.2	0.801
FI				
F0-F3	< 2.1	34.4	82.2	0.556
F4	> 3.3	92.3	78.1	0.826
API				
F0-F3	<2.5	58.4	70	0.624
F4	>2.5	60	78.1	0.578
Pohl Score				
F0-F3	0	58.4	30	0.499
F4	1	78.1	38.1	0.599
FCI				
F0-F3	< 0.131	57.4	37	0.499
F4	> 1.25	88.1	78.1	0.867

(AAR=AST to ALT Ratio, APRI=AST to Platelet index, FI=Fibrosis Index, FIB-4=Fibrosis-4, FCI=Fibrosis Cirrhosis Index, API=Age to Platelet index)

specificity along with cutoff points were calculated **(table 9) & (figure 2)**.

DISCUSSION

Hepatitis C leads to liver cirrhosis and liver cancer. Approximately 30 years is the mean infection time for origin of cirrhosis, with people in age group of 10-50 years on risk of cirrhosis.[19] Spread of fibrosis in hepatic tissue is considered as a gold standard for confirmation of cirrhosis. Several indexes are available for predicting the onset of cirrhosis but not any exclusive and dependable method has yet been established for assessment of fibrosis.

Most common form of hepatitis in Pakistan is genotype is 3a and 2nd most common form is 1a and exactly corresponding of what we observed in this study. Genotype 3a was in 86% of patients while rest of them were diagnosed with genotype 1a earlier stages of fibrosis (F1-F2) were diagnosed among much younger patients as compared to later stage of fibrosis (F3-F4) which was present among older people.[3,20-22] The results of this study supported the previous researches that subjects with mild fibrosis were found to be younger than the intermediate and advanced fibrosis stages and gender has nothing to do with stage of fibrosis.[23-25]

Our study's results back the latest recommendations by EASL to apply non-invasive tests (NITs) as first line tests in prognostication of liver fibrosis.[19,22-23,26] According to our conclusions and these new recommendations, liver biopsy is needed only if redundant non-invasive tests show dissension. Blood markers can be used to predict cirrhosis and advanced stages of fibrosis and should be used if TE is not available or cost effective to patient or when diagnostic yield is constrained as in obese patients.[13,24]

AAR, at cutoff value <1 sensitivity was 62.5% & specificity was 41.9% and AUC was 0.377 for predicting F0-F3. At cutoff value > 1, sensitivity and specificity were 62.8% & 37.6% with AUC= 0.412 for F4. At cutoff value <0.5 APRI predicted F0-F3 with 56.2% sensitivity and 68.05% specificity with AUC = 0.546. At cut off value >1.5, F4 was predicted by 74.6%sensitivity and 87.6% specificity having AUC=0.864. FIB-4 was invented by Sterling in 200620 and at cutoff value <1.45, F0-F3 was having sensitivity 51%& specificity

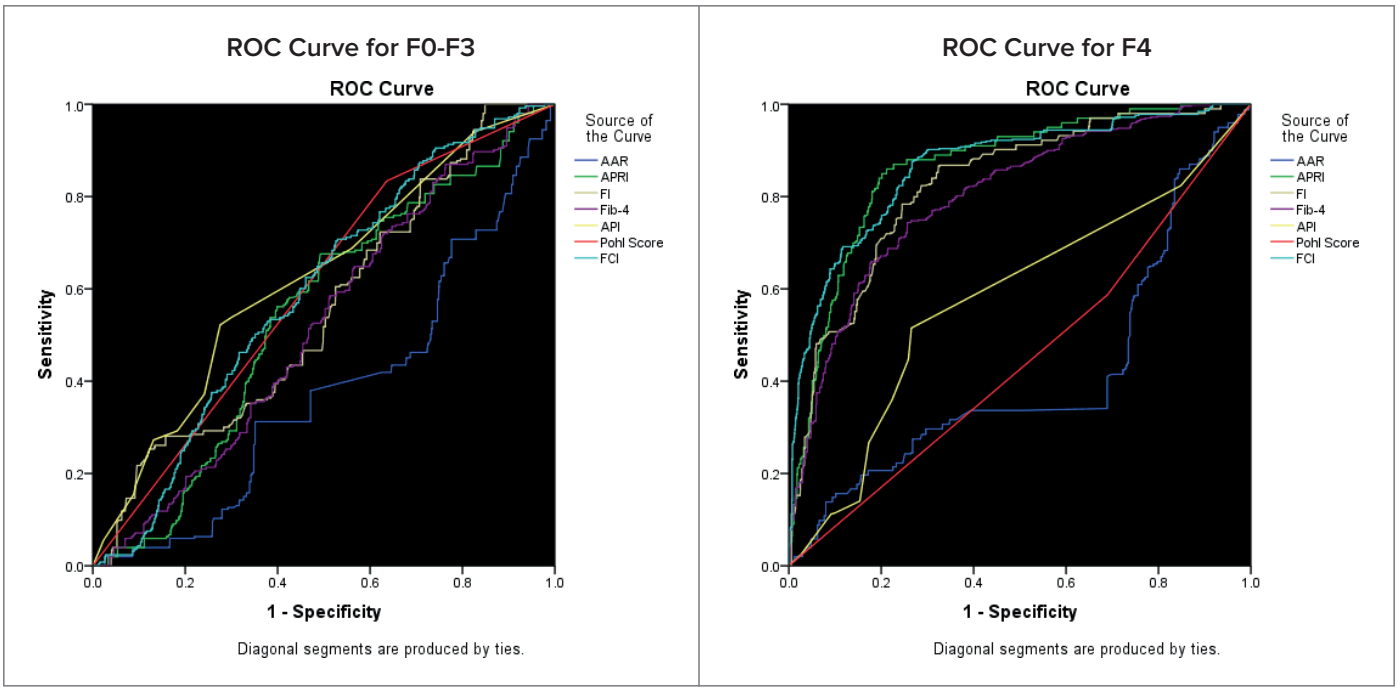

Figure 2: ROC Curve analysis for validation of serum ALT/AST ratio, AST platelet ratio APRI, Fibrosis 4, Fibrosis index, API, Pohl score and FCI

65.4% with area under curve (AUC) =0.521. At cut-off value > 3.25 for F4 sensitivity was 53.2% and specificity 72.3% with AUC=0.801. FI was invented by Ohta in 200616 and at cut-off value <2.1, sensitivity for prediction of F0-F3 was 82.2% and specificity 34-4% having an area under curve (AUC) equals 0.556. At cut-off value >3.3, sensitivity was 78.1% & specificity 92.3% with AUC =0.826 for predicting F4. API at cutoff value <2.5 ,sensitivity for prediction of F0-F3 was 70% and specificity for predicting F0-F3 was 58.4% and area under curve(AUC) equals 0.624 . At cutoff value >2.5 for prediction of F4, sensitivity was 78.1%& specificity 60% with AUC=0.578. Pohl Score were not found to be a good index to stage fibrosis. At cutoff value <0 for predicting F0-F3 ,sensitivity was 30% & specificity was 58.4% and area under curve(AUC) equals 0.499 .At cut-off value > 1 , sensitivity was 38.1%& specificity was 78.1% with AUC=0.549 for F4. FCI was found to be a good test in predicting cirrhosis than non-cirrhotic stages. At cutoff value <0.131, sensitivity & specificity for predicting F3 were 37% & 57.4% and AUC was 0.529. At cutoff value > 1.25, sensitivity was 78.1% & specificity was 88.1% with AUC =0.867 for F4.

CONCLUSION

Readily available and cheap serum indices AAR, APRI, FI, FIB-4, API, Pohl score and FCI predicted late stages of fibrosis in co-relation to the costly and less accessible Fibro scan with considerable accuracy as per their design.

REFERENCES

1. Attaullah S, Khan S, Ali I. Hepatitis C virus genotypes in Pakistan: a systemic review. Virology journal. 2011 Dec;8(1):433.
2. Thampanitchawong P, Piratvisuth T. Liver biopsy: complications and risk factors. World journal of gastroenterology. 1999 Aug 15;5(4):301.
3. Ahmad W, Ijaz B, Javed FT, Gull S, Kausar H, Sarwar MT, Asad S, Shahid I, Sumrin A, Khaliq S, Jahan S. A comparison of four fibrosis indexes in chronic HCV: development of new fibrosis-cirrhosis index (FCI). BMC gastroenterology. 2011 Dec;11(1):44.
4. Schmid P, Bregenzer A, Huber M, Rauch A, Jochum W, Müllhaupt B, Vernazza P, Opravil M, Weber R, Swiss HIV Cohort Study. Progression of liver fibrosis in HIV/HCV co-infection: a comparison between non-invasive assessment methods and liver biopsy. PloS one. 2015 Sep 29;10(9):e0138838.
5. Crossan C, Tsochatzis EA, Longworth L, Gurusamy K, Davidson B, Rodríguez-Perálvarez M, Mantzoukis K, O'Brien J, Thalassinos E, Papastergiou V, Burroughs A. Cost-effectiveness of non-invasive methods for assessment and monitoring of liver fibrosis and cirrhosis in patients with chronic liver disease: systematic review and economic evaluation.
6. European Association for Study of Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. Journal of hepatology. 2014 Feb;60(2):392.
7. European Association for the Study of the Liver. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. Journal of hepatology. 2015 Jul 1;63(1):237-64.
8. Adams LA, Sterling RK. Developing a new algorithm to diagnose advanced liver fibrosis: A lift or a nudge in the right direction? Journal of hepatology. 2017 Jun 1;66(6):1111-3.
9. Fallatah HI. Noninvasive biomarkers of liver fibrosis: an overview. Advances in Hepatology. 2014;2014.

10. Boursier J, De Ledinghen V, Zarski JP, Fouchard-Hubert I, Gallois Y, Oberti F, Calès P, multicentric groups from SNIFF 32, VINDIAG 7, and ANRS/HC/EP23 FIBROSTAR studies. Comparison of eight diagnostic algorithms for liver fibrosis in hepatitis C: new algorithms are more precise and entirely noninvasive. Hepatology. 2012 Jan;55(1):58-67.
11. Zarski JP, Sturm N, Guechot J, Paris A, Zafrani ES, Asselah T, Boisson RC, Bosson JL, Guyader D, Renversez JC, Bronowicki JP. Comparison of nine blood tests and transient elastography for liver fibrosis in chronic hepatitis C: the ANRS HCEP-23 study. Journal of hepatology. 2012 Jan 1;56(1):55-62.
12. Giannini C, Brechot C. Hepatitis C virus biology. Cell death and differentiation. 2003 Mar 25;10(S1):S27.
13. Alter MJ. Epidemiology of hepatitis C. Hepatology. 1997 Dec;26(S3):62S-5S.
14. Raja NS, Janjua KA. Epidemiology of hepatitis C virus infection in Pakistan. Journal of Microbiology Immunology and Infection. 2008 Feb 1;41(1):4.
15. Idrees M, Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC infectious diseases. 2008 Dec;8(1):69.
16. El-Serag HB. Hepatocellular carcinoma and hepatitis C in the United States. Hepatology. 2002 Nov;36(5B):s74-83.
17. National Institute of health Consensus Development Conference Statement: Management of Hepatitis C: 2002. Hepatology 2002, 36:973-977.
18. Silva Junior RG, Fakhouri R, Nascimento TV, Santos IM, Barbosa LM. Aspartate aminotransferase-to-platelet ratio index for fibrosis and cirrhosis prediction in chronic hepatitis C patients. Brazilian Journal of Infectious Diseases. 2008 Feb;12(1):15-9.
19. Parise ER, Oliveira AC, Figueiredo-Mendes C, Lanzoni V, Martins J, Nader H, Ferraz ML. Noninvasive serum markers in the diagnosis of structural liver damage in chronic hepatitis C virus infection. Liver International. 2006 Nov;26(9):1095-9.
20. Azzari C, Resti M, Moriondo M, Ferrari R, Lionetti P, Vierucci A. Vertical transmission of HCV is related to maternal peripheral blood mononuclear cell infection. Blood. 2000 Sep 15;96(6):2045-8.
21. Kowala-Piaskowska A, Mozer-Lisewska I, Figlerowicz M, Służewski W. Influence of the presence of HCV-RNA in peripheral blood mononuclear cells on the clinical course of chronic hepatitis C in children. European journal of epidemiology. 2007 May 1;22(5):343-8.
22. 22. Castera L, Pinzani M. Non-invasive assessment of liver fibrosis: are we ready? The Lancet. 2010 Apr 24;375(9724):1419-20.
23. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis relationship to cirrhosis. Gastroenterology. 1988 Sep 1;95(3):734-9.
24. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003 Aug 1;38(2):518-26.
25. Ohta T, Sakaguchi K, Fujiwara A, Fujioka SI, Iwasaki Y, Makino Y, Araki Y, Shiratori Y. Simple surrogate index of the fibrosis stage in chronic hepatitis C patients using platelet count and serum albumin level. Acta Medica Okayama. 2006 Apr 1;60(2):77.
26. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. New England Journal of Medicine. 2000 Apr 27;342(17):1266-71.

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1a. Miner P et al. Gastric acid control with Esomeprazole, Lansoprazole, Omeprazole, Pentaprazole & Rabiprazole: A five way cross over study. AM J Gastroenterol. 2003;98: 2616-20
1b. J. Dent Review article: Pharmacology of Esomeprazole & comparison with Omeprazole. Ailment Pharmacol Ther 2003; 17 (suppl. 1): 5-9
2a. The state of Helicobacter pylori infection in south Asian countries: Anigma Hossain et al. Int J Gastroenterol Hepatol Transpl Nutr 2016;1(i): 11-14
2b. Laine L, et al. AM J Gastroenterol 1998; 93: 2016-12
3. Scheiman JM , Yeomans ND , Talley NJ et al. Prevention of ulcers by esomeprazole in at-risk patients using non-selective NSAIDs and COX-2 inhibitors. Am J Gastroenterol 2006 ; 101 : 701-10.
4. Nexium Prescribing information, Wilmington, DE; Astrazenica Pharmaceuticals LP; March 2014

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