Original Article

Hepatic Overlap Syndrome: Glass of Autoimmune Mimicry - Half Empty and Half Filled

Hussain Haroon, Pyar Ali Fazel, Ahsan Mobin, Muhammad Naeem, Shirjeel Hussain, Karim Ullah Makki

ABSTRACT

OBJECTIVE: To unfold the iceberg entity of hepatic overlap syndrome among patient with established chronic liver diseases.

METHODOLOGY: Forty-one patients were referred from the remote areas of Sindh province during March 2008 to August 2010 to Department of Internal Medicine at Civil Hospital, Karachi, Pakistan. These patients were unresponsive to the treatment(s) being prescribed. The complete biochemical, clinical, serological and histopathological profiles of these patients was assessed. The Chazouillères criterion was used to diagnose the overlap syndrome cases (according to which an AIH-PBC overlap syndrome would be accepted when 2 out of 3 criteria each of AIH or PBC is fulfilled). Data were analyzed through SPSS version 16.0. Frequencies and means were calculated. Serological markers were correlated against biopsy patterns by Pearson's, Cramer's V test, Mantel-Haenszel Test to measure the odd of getting the particular biopsy pattern and 2-sided significance level <0.10 was taken as significant.

RESULTS: Out of 41 patients, 23 had AIH or PBC (56.09%) and the other 18 (43.90%) were having CLD secondary to Wilson's disease (9 patients), fatty liver (4 patients), Hepatitis B (3 patients) and hepatitis C (2 patients). Further workup in 23 patients showed that 10 out of 23 were having AIH/PBC overlap. Fatigue was the most common clinical symptom among Overlap syndrome patients (80%). 3 patients were having Hashimoto's thyroiditis and one male was diagnosed with SLE.

CONCLUSION: We emphasize and recommend for detail investigations to consider such entity for autoimmune chronic liver disease, where initial laboratory investigations do not support the exact diagnosis among CLD patients. Since no work has been done so far on overlap syndrome in our set up, we suggest multicentre prevalence surveys to develop proper treatment guidelines for Indo-Pakistan population.

KEYWORDS: Hepatic Overlap Syndrome; Chronic Liver Disease; Chazouillères criterion; Hashimoto's thyroiditis.

INTRODUCTION

Our current understanding of the autoimmune liver disease mechanism proposes that it is a disorder of the immune tolerance possibly influenced by complex and multi-factorial predictors. The auto destruction can be targeted against different liver cell populations such as hepatocytes and/or bile duct epithelium resulting in autoimmune hepatitis (AIH) or primary biliary cirrhosis (PBC) and possibly primary sclerosing cholangitis (PSC) [1, 2].

Primary biliary cirrhosis and autoimmune hepatitis are the two main immune-mediated liver diseases. Generally these two may be differentiated easily on the basis of clinical, biochemical, serological, and histological findings. Overlap Syndrome includes various forms of autoimmune hepatobiliary diseases such as AIH, PBC, PSC, and autoimmune cholangitis (AIC) coexisting in an individual. Occasionally, the normal distinctive features of overlap syndrome and classification of a given patient's chronic liver disease is difficult. Series of such patients with PBC-AIH overlap syndrome were reported in the 1970s[3].

So far no standard diagnostic criteria for overlap syndromes have been achieved[4] and this area has not been explored in detail in our region.

Our aim was to unfold the iceberg entity in patients with established liver diseases and to emphasize the importance of thorough investigations where initial labs do not support the exact diagnosis.

MATERIALS AND METHODS

This study was conducted from March 2008 till August 2010 at department of medicine, Dow Medical College, Dow University of health sciences, Karachi, Pakistan.

Forty one cases were referred from remote areas of Sindh province for their assessment as all these patients were unresponsive to therapy either due to non-compliance, improper treatment, or improper diagno-

ses.
All the patients from either sex greater than 12 years of age with established autoimmune liver disease on serological basis or those with chronic liver disease of unknown etiology were included.
All the liver diseases which were secondary to pregnancy, drugs, alcohol, or non alcoholic steatohepatitis were excluded from the study. Before inclusion verbal as well as written consent was taken from patients.
Forty-one patients met the inclusion criteria and out of 41 patients, 23 were diagnosed at referral institutes as either AIH or PBC on basis of serology only and the remaining 18 diagnosed chronic liver disease with unexplained rise in liver enzymes and were negative for hepatitis B or C on basis of ICT kit and no further workup was done so far. In order to diagnose those 18 patients, detailed workup was done including history, clinical examination, serum and urine levels of ceruloplasmin, liver function tests, ultrasound abdomen and anti HCV and HbsAg on ELISA.
Their detailed history and clinical examination of all the patients was done by two separate authors at two different times to eliminate the chances of bias. Their degree of agreement (κ) on findings of clinical examination was 0.95. History of onset of illness, acute precipitating events, blood transfusion, surgical intervention, menstruation abnormalities, drug history, presence of extra-hepatic features were specifically noted. Family history of autoimmune disease was also noted as well as alcohol history was sorted out.
Lab investigations were again sent to refresh the diagnosis. Baseline tests like complete blood count along with bleeding and clotting profile was sent. All patients underwent biochemical evaluation using standard automated techniques. Liver function test and serum protein Albumin: Globulin ratio, protein electrophoresis were done along with ultrasound abdomen. In Protein electrophoresis, IgG and IgM levels were also sent, IgG levels > 2 times the upper normal limit (UNL) and IgM levels > 1.5 times the upper normal limit were considered as significant. Serum Alanine aminotransferase levels >5 times the UNL and serum Alkaline phosphatase levels > 2 times the UNL were considered significant.
Serological tests for auto antibodies ANA profile (ANA, ASMA, Anti-LKM1, and AMA) were done in all patients and their titers checked through indirect immunofloresence. Titer above 1:80 for ANA, ASMA and Anti-LKM1 were taken as positive. Titer above 1:40 for AMA was considered significant. Anti ds-DNA antibodies was also sent in some patients with clinical significant features suggestive of SLE. Antibodies against thyroid were also measured.
Percutaneous liver biopsies were done in all selected 23 patients after having all biochemical and serological assessment to finalize the diagnoses and to recommend appropriate treatment. The procedure was done in accordance with AASLD guidelines 2009[23]. The biopsies were interpreted by two separate histopathologists not known to each other and the degree of agreement was found to be 0.789. The aspirated sample was fixed in 10% formalin and then was assessed under light microscope with 5 histological stains viz., hematoxylin and eosin, Masson's trichome stain, reticulin, periodic acid Schiff (PAS) and elastin Von geisen. Ishak modified HAI scores[5] were applied for diagnosing the specimens.
We used the diagnostic criteria proposed by chazouillères et al in 1998[3], according to which an AIH-PBC overlap syndrome would be accepted when 2 out of 3 criterias each of AIH or PBC is fulfilled.
AIH (2 out of 3 criteria)
Serum ALT levels > 5 × UNL
Serum IgG levels > 2 × UNL or a positive test for ASMA (Anti smooth muscles antibodies)
Liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis.
PBC (2 out of 3 criterias)
Serum ALP levels > 2 × UNL or γ-glutamyl transpeptidase levels > 5 × UNL
Positive test for anti mitochondrial antibodies (AMA)
Liver biopsy showing florid bile duct lesions.
All the data were collected through a structured questionnaire which was filled by authors themselves. All the data was entered into SPSS ver.16.0. Frequencies were calculated and mean was applied for calculation of ages and duration of symptoms. Every variable in the study was correlated with the liver biopsy pattern. Correlations were first determined through Pearson's and then post tested by Cramer's V test to determine strength of association between variables. Positive and negative serological markers were correlated against biopsy pattern on contingency tables by Mantel-Haenszel Test to measure the odds of getting the particular biopsy pattern. Correlations were estimated on a scale where 2 sided significance levels <0.10 was taken as level of significance.

RESULTS

Age ranges from 25 to 55 years in 41 patients. Out of them 23 (56.10%) had PBC, AIH or OS and 18 (43.90%) were stratified after investigations into Wilsons disease (9 patients), fatty liver (4 patients), hepatitis B positive on ELISA (3 patients) and hepatitis C positive on ELISA (2 patients).
The 23 patients which were initially diagnosed as AIH or PBC were further worked up for OS.
4 out of 10 (40%) PBC patients had histopathological pattern favoring AIH i.e. PBC/AIH overlap; while 6 out of 12 AIH patients (50%) had PBC pattern on liver

biopsy and were labeled as AIH/PBC overlap (one male patient having PBC also having SLE on serological basis and on liver histopatholgically AIH (PBC/AIH with SLE)).

Being the main focus of the study we would explain the relevant results of 10 patients of our study. The clinical features on examination which were found in 10 patients are shown in **Table I**.

Fatigue was the most common symptom (80%) followed by jaundice (70%), hepatosplenomegaly (70%), pedal edema (50%), anemia (40%), itching (40%), arthralgia (40%), shrunken liver (30%), goiter (20%) and rash (10%).

Regarding other autoimmune diseases, SLE was present in one male patient and hashimoto thyroiditis in three patients. Hepatitis C and Hepatitis B viral markers were negative in all 10 patients based on ELISA. All the features are shown in **Table II**. The schematic diagram of diagnosis of patients is presented in **Figure I**.

TABLE I: CLINICAL FEATURES OF PATIENTS WITH OS

Clinical Features	Frequency (%)
Fatigue	8 (80)
Jaundice	7 (70)
Hepatosplenomegaly	7 (70)
Pedal Edema	5 (50)
Anemia	4 (40)
Itching	4 (40)
Arthralgias	4 (40)
Shrunken Liver	3 (30)
Goitre	2 (20)
Rash	1 (10)

TABLE II: FEATURES OF 10 PATIENTS DIAGNOSED WITH OS

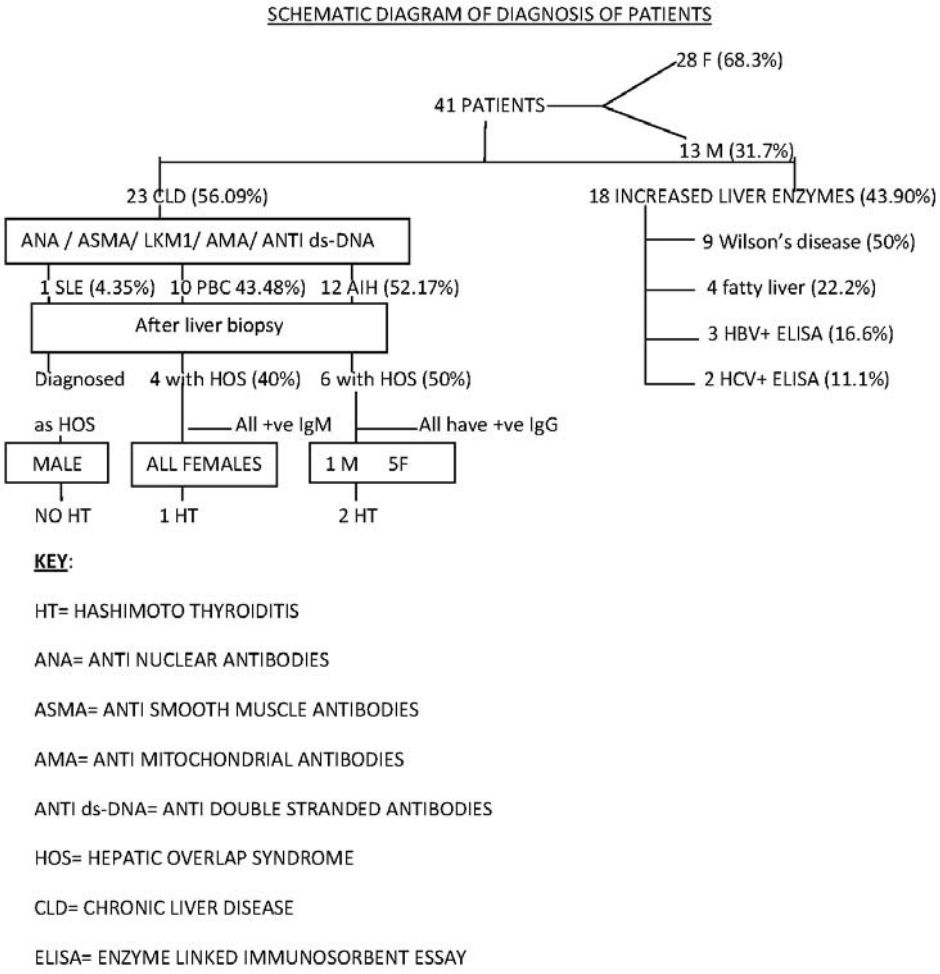
Age (yr)	Sex	Symptoms duration (Years)	Co morbidities	Thyroid antibodies	ANA profile	ANTI ds-DNA	Viral markers	IgG	IgM	Liver biopsy
24	F	2	-	-	ANA/ ASMA +	-	-	+	-	Consistent with PBC
25	F	3	Goiter/ Hashimoto thyroiditis/ Hypothyroidism	+	ANA/ ASMA +	-	-	+	-	Consistent with PBC
35	F	1	Goiter/ Hashimoto thyroiditis/ Hypothyroidism	+	AN-TILKM 1 +	-	-	+	-	Consistent with PBC
38	F	2.5	-	-	ANA +	-	-	+	-	Consistent with PBC
31	M	4	-	-	ANA/ ASMA +	-	-	+	-	Consistent with PBC
36	F	1.5	Hashimoto thyroiditis/ Hypothyroidism	+	AMA +	-	-	-	+	Consistent with AIH
50	F	6	-	-	AMA +	-	-	-	+	Consistent with AIH
37	F	3.5	-	-	AMA +	-	-	-	+	Consistent with AIH
40	M	2	SLE	-	ANA/ ASMA +	+	-	+	-	Consistent with PBC
36	F	2	-	-	AMA +	-	-	-	+	Consistent with AIH

TABLE III: SIGNIFICANCE OF CORRELATION OF SEROLOGY WITH HISTOPATHOLOGICAL PATTERNS

	Biopsy Patterns		
Anti Bodies	**Pearsons correlation 2 sided-significance**	**Cramers V 2 sided-significance**	**Mantel-Haenszel Test 2 sided-significance**
ANA	0.010*	0.010*	0.066*
Anti-LKM 1	0.389	0.389	0.838
AMA	0.002*	0.002*	0.018*
Anti ds-DNA	0.389	0.389	0.838
ASMA	0.054*	0.054*	0.076*

*= *SIGNIFICANT VALUES*

FIGURE I: DIAGNOSTIC SCHEME OF PATIENTS



DISCUSSION

PBC and AIH are the most frequent autoimmune hepatopathies in recent epidemiologic studies in Europe and United states with female predominance in both AIH (80%) and PBC (90-95%)[6-8], similarly most of our patients were female too.

So far no consensus has been reached on the definition of overlap and outlier syndromes in hepatology[4] but the term overlap syndrome has been introduced to the field of hepatology to describe variant forms of autoimmune hepatitis (AIH) which present with characteristics of AIH and Primary biliary cirrhosis (PBC)

or primary sclerosing cholangitis (PSC). There is still controversy whether these overlap syndromes form distinct entities or are only variants of the major autoimmune hepatopathies. Standardization of diagnostic criteria have not been proposed so far as these syndromes are rare.[9]

Overlap syndrome should always be considered once an autoimmune liver disease has been diagnosed[10]. The term overlap syndrome is used primarily to describe variants forms of autoimmune liver disease that present with both cholestatic and hepatitic features that do not fit readily into the usual diagnostic categories and which generally have overlapping characteristics of AIH+PBC or AIH+PSC.[11]

In order to avoid any disparity in diagnosis of overlap syndrome we use scrupulous criteria for diagnosing PBC and AIH described elsewhere [12, 13] so that our cases should be as genuine as possible because so far no single diagnostic criterion is yet trustworthy. Up to 10 % AIH or PBC patients may belong in this overlap category, but because reported criteria vary among studies, comparison is difficult. However, this problem was resolved up to some extent in the diagnostic criteria set up by Chazouillères O et al[3], in which two out of the three criteria for AIH and PBC were required. We used the same criteria for OS patients. 10 out of 41 patients (24.39%) in our study were diagnosed with overlap syndrome; while two previous studies have shown the evidence of AIH-PBC overlap in 8% of 199 patients and 9% of the 130 patients. In the later study an AIH-PBC overlap syndrome was accepted when 2 or 3 criteria for PBC and AIH were fulfilled; although these diagnostic criteria for an AIH-PBC overlap syndrome are not validated and their sensitivity has not been established, they provide a diagnostic template that can be consistently applied.[9]

The first case series of such patients were presented almost 33 -34 years ago but still the dilemma regarding its etiology is not completely apprehended, many problems are still unresolved.[14-16]

Although it may be difficult to distinguish AIH from PBC on histologic grounds, accurate diagnosis is important because treatment for AIH differs from that for PBC. Difficulties arise because the portal inflammatory infiltrate of PBC often contains numerous plasma cells, and infiltration of bile duct epithelium by lymphocytes is not uncommon in AIH, if looked for, and some degree of bile duct injury is often present[11]; while the agreement of 78.9% among the histopathologists in our study have minimized the observer bias. Autoimmune hepatitis can be distinguished from primary biliary cirrhosis with confidence in 92% of cases.[17, 18]

We correlate the strength of association of serological markers with the particular biopsy pattern statistically as shown in table 3. ANA profile was positive in 5 out of 6 patients who were having histopathological patterns of PBC. Our results found that ANA presence in this type of pattern is statistically very significant with positive correlation among the two variables but still this autoantibody is not the helpful marker of overlap syndrome because ANA are found in high rates in PBC patients. [24,25] ANA represent the least specific serum auto antibodies for the diagnosis of chronic liver diseases and are also found in 30% of elderly healthy controls, 10% of pregnant women, and 30% of patients with malignancies. Serum ANA in patients with PBC are not a marker of AIH/PBC overlap syndrome, but often found in PBC patients without further signs of AIH [19].

Apart from that, no significant correlation was seen between Anti-LKM1 and biopsy patterns. AMA was positive in 4 out of 10 patients who were serologically diagnosed as PBC patients. Statistically this was well correlated with the histopathological patterns, the most specific features of PBC is the presence of AMA in the serum of 90%-95% of patients affected[12]

Hashimoto thyroiditis was present in 3 out of 10 patients and SLE in one male patient, so overall 40 % of them were affected with concomitant autoimmune disorders. In our study only one patient had concomitant SLE, who was male. Liver involvement in patients with connective tissue diseases has been well documented but is generally considered rare. Although hepatic steatosis and abnormal results on biochemical liver function tests are the most common hepatic abnormalities associated with connective tissue diseases, other less frequent abnormalities have been noted, such as nodular regenerative hyperplasia, portal vein obliteration and portal hypertension, features of primary biliary cirrhosis, and rarely portal fibrosis with abnormal lobular architecture[20].

The term Overlap syndrome should not be used in patients having AIH and chronic liver diseases like hepatitis C [21]. Therefore, we exclude all the patients who were diagnosed having wilson's, hepatitis B or C and fatty liver. None of the patient in diagnosed overlap syndrome was found positive for Hepatitis B or C.

In summary, the clinical features of PBC-AIH overlap syndrome are in various forms. It has the clinical and

histopathological characteristics of both PBC and AIH. Accurate and prompt diagnosis of overlap syndrome should be based on the clinical presentation, biochemical and immune indices and hepatic pathological changes. Various overlap forms of autoimmune hepatitis are not uncommon. Recognition of them is important in assessing common pathogenic mechanisms, developing effective treatment strategies and refining classification schemes.[22]

CONCLUSION

Finally we emphasize and recommend thorough evaluation to consider such entity while dealing with autoimmune chronic liver disease or other ensuing autoimmune systemic diseases. Long term follow-up is needed to see disease progression as one disease can entirely overshadow the existence of other over the period of time. Since no work has been done so far on overlap syndrome in our set up, we aim to have a multicenter prevalence surveys so that proper treatment guidelines can be made for our population.

COMPETING INTERESTS

This work did not benefit from any external funding and the authors have no conflicts of interest to report.

REFERENCES

1. Abdo AA, Bain VG, Kichian K, Lee SS. Evolution of autoimmune hepatitis to primary sclerosing cholangitis: a sequential syndrome. Hepatology 2002; 36: 1393-1399.
2. Woodwards J, Neuberger J. Autoimmune overlap syndrome. Hepatology 2001; 33: 994-1002.
3. Chazouillères O, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary Biliary Cirrhosis-Autoimmune Hepatitis Overlap Syndrome: Clinical features and response to therapy. Hepatology 1998; 28(2): 296-301.
4. Beuers U. Hepatic overlap syndromes. J Hepatol 2005; 42: 93-99.
5. Brunt EM. Grading and Staging the histopathological lesions of chronic hepatitis: The Knodell Histology Activity Index and Beyond. Hepatolgy 2000; 31(1):241-6.
6. Kim WR, Lindor KD, Locke GR 3rd, Therneau TM, Homburger HA, Batts KP, et al. Epidemiology and natural history of primary biliary cirrhosis in a US community. Gastroenterology 2000; 119: 1631-36.
7. Prince MI, James OF. The epidemiology of primary biliary cirrhosis. Clin Liver Dis 2003; 7: 795-819.
8. Boberg KM. Prevalence and epidemiology of autoimmune hepatitis. Clin Liver Dis 2002; 6: 635-47.
9. Rust C, Beuers U. Overlap syndromes among autoimmune liver diseases. World J Gastroenterol 2008; 14(21): 3368-73
10. Czaja AJ. The variant forms of autoimmune hepatitis. Ann Intern Med 1996; 125: 588-598.
11. Washington MK. Autoimmune liver disease: overlap and outliers. Modern Path 2007; 20: 15-30.
12. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Eng J Med 2005; 353: 1261-73.
13. Krawitt EL. Autoimmune hepatitis. N Eng J Med 2006; 354: 54-66.
14. Geubel AP, Baggenstoss AH, Summerskill WHJ. Responses to treatment can differentiate chronic active liver disease with cholangitic features from the primary biliary cirrhosis syndrome. Gastroenterology 1976; 71: 444-449.
15. Kloppel G, Seifert G, Lindner H, Dammermann R, Sack HJ, Berg PA. Histopathological features in mixed types of chronic aggressive hepatitis and primary biliary cirrhosis. Virchows Arch A Pathol Anat Hiatopathol 1977; 373: 143-60.
16. Shouval D, Levij IS, Eliakim M. Chronic active hepatitis with cholestatic features. Am J Gastroenterol 1979; 72: 542-50.
17. Kenny RP, Czaja AJ, Ludwig J, Dickson ER. Frequency and significance of antimitochondrial antibodies in severe chronic active hepatitis. Dig Dis Sci 1986; 31: 705-11.
18. Czaja AJ, Carpenter HA, Manns MP. Antibodies to soluble liver antigen P45011D6 and mitochondrial complexes in chronic hepatitis. Gastroenterology 1993; 105: 1522-8.
19. Kanzler S, Bozkurt S, Herkel J et al. Presence of SLA/LP autoantibodies in patients with primary biliary cirrhosis as a marker for secondary autoimmune hepatitis (overlap syndrome). Dtsh Med Wochenschr 2001; 2126: 450-56.
20. Youssef WI, Tavill AS. Connective tissue diseases and the liver. Clin Gastroenterol 2002; 35(4): 345-9.
21. Pawlotsky JM, Ben Yahia M, Andre C, Voisin MC, Intrator L, Roudot-Thorval F, et al. Immunological disorders in C virus chronic active hepatitis: A prospective case-control study. Hepatology 1994; 19: 841-48.
22. Joshi S, Cauch Dudek K, Wanless I, Linder K,

Jorgensen R, Batt K et al. Primary Biliary cirrhosis with additional features of Autoimmune hepatitis: Response to therapy with UDCA. Hepatology 2002; 35: 409-13.
23. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. Hepatology 2009; 49 (3): 1017-44.
24. Terjung B, Spengler U. Role of autoantibodies for the diagnosis of chronic cholestatic liver diseases. Clin Rev Allergy Immunol 2005; 28: 115-33.
25. Invernizzi P, Selmi C, Ranftler C, Podda M, Wesierska-Gadek J. Antinuclear antibodies in primary biliary cirrhosis. Semin Liver Dis 2005; 25: 298-310.

AUTHOR AFFILIATION:

Dr. Hussain Haroon *(Corresponding Author)*
Assistant Professors, Department of Internal Medicine
Dow University of Health Sciences/Civil Hospital
Karachi, Sindh-Pakistan.
Email: lillahone@hotmail.com

Dr. Pyar Ali Fazel
Assistant Professors, Department of Internal Medicine
Dow University of Health Sciences/Civil Hospital
Karachi, Sindh-Pakistan.

Dr. Ahsan Mobin
Registrar, Department of Internal Medicine
Dow University of Health Sciences/Civil Hospital
Karachi, Sindh-Pakistan.

Dr. Muhammad Naeem
Final Year Student
Dow University of Health Sciences
Karachi, Sindh-Pakistan.

Dr. Shirjeel Hussain
Chief Resident, Department of Internal Medicine
Dow University of Health Sciences/Civil Hospital
Karachi, Sindh-Pakistan.

Dr. Karim Ullah Makki
Professor, Department of Internal Medicine
Dow University of Health Sciences/Civil Hospital
Karachi, Sindh-Pakistan.