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Contains 80 pages from 81 to 160 (inclusive of all advertisements)

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Corrigendum

JIACM 2023 Jan-March*, 2023; Vol. 24 (1): Page 49-57*. The Review Article - **Approach to A Patient with Tremor.** *Name of the first author in this article is to be read as Kamakshi Dhamija insted of Kamikashi Dhamija.*

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Editorial/Mailing Address

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ORIGINAL ARTICLE

JIACM 2023; 24 (2): 86-90

Association of HbA1c with HRCT Thorax among Diabetic and Non-Diabetic COVID Patients

Sandhya Gautam, Vivek Yadav**, Chhaya Mittal****, Snehlata Verma***, Yasmeen Usmani******

Abstract

Introduction and objectives: *Diabetes mellitus is a multiorgan disease which is slowly growing as a major disease burden on world health. COVID-19 is an active deadly pandemic that is still threatening human lives. It has been observed that the interaction of these two slow and rapid pandemics together leads to severe mortality and morbidity. The study was done to assess the severity of COVID disease in diabetic and non-diabetic patients according to HRCT severity score and duration of hospital stay.*

Methods: *This retrospective single centred case control study was conducted in SVBP hospital and LLRM Medical College, Meerut in admitted patients during September 2020 to May 2021 of COVID-19 disease. The calculated sample size came out to be 81. So, 81 COVID positive diabetic patients were enrolled and an equal number of non-diabetic Covid patients were taken as control. In our study, association of HbA1c with CT severity score and their duration of hospital stay was studied.*

Results: *It was observed that the HRCT severity score was more in the uncontrolled than in controlled diabetic group of Covid patients. The association of HRCT severity score with various HbA1c levels among diabetic and non-diabetic patients was significant with p value of 0.029. Similarly, the duration of hospital stay was associated with HRCT severity score significantly. HRCT severity score was raised in both the groups diabetic and non-diabetic with significant p value of 0.001 and 0.008 respectively.*

Conclusion: *COVID-19 disease with diabetes is a lethal combination. Association of HRCT severity score is significantly associated with increasing HbA1c levels rather than diabetes status. Poor glycaemic control can lead to severe disease related complications. Duration of hospital stay was significantly associated with HRCT severity score among both diabetic and non-diabetic Covid patients. Thus, in known diabetic patients with strict glycaemic control, early CT-imaging of the disease severity can have better outcomes in COVID pandemics.*

Key words: *COVID-19 disease, diabetes mellitus (DM), HRCT severity score.*

Introduction

COVID-19 disease was a pandemic caused by the novel SARs-COV-2 discovered in December 2019 in Wuhan, China. COVID-19 infection was highly contagious and communicable which had spread around the world, within weeks. Amongst COVID-19 infected patients, majority of the patients had milder symptoms, but few patients had severe illness including respiratory distress as major killer. COVID-19 disease caused huge death tolls[1] in elderly and co-morbid patients. In India, from 3 January 2020 to 6:07 pm CEST, 31 May 2023, there have been 44,990,278 confirmed cases of COVID-19 with 5,31,867 deaths, reported to WHO[2]. Diabetes mellitus is a chronic multi-system disease, which is caused by inadequate or inappropriate insulin synthesis and metabolism in the body. The resultant hyperglycaemia causes serious damage to various organ systems of the body and results in dysfunction of the immune system. This leads to failure of protection from pathogenic invasions and infections[3]. Diabetes mellitus forms a major portion of non-communicable disease and causes large mortality and morbidity worldwide. Diabetic people are more likely to develop serious complications with COVID-19 infection[4]. Therefore, this study was done to see the association of HRCT severity score in COVID-19 patients with HbA1c. HRCT thorax is one of the main diagnostic tools for COVID-19 severity grading as well as prognosis. On HRCT thorax scan, infected individuals generally showed a multifocal or mono-focal involvement of ground-glass opacity (GGO)[5]. HRCT thorax had been widely accepted for prognostication of COVID-19 pneumonia[6]. Several remarkable chest CT findings had been reported in more than 70% of RT-PCR test–proven COVID-19 cases, including ground-glass opacities, vascular enlargement, bilateral abnormalities, lower lobe involvement, and posterior predilection[7]. The severity of the lung involvement on the CT correlates with the severity of the disease. HRCT thorax imaging severity scores of >18 have been linked to higher risks of death and have been found to be better predictor of death among diabetic COVID-19

**Professor, **Junior Resident, ****Associate Professor, Department of Medicine, ***Associate Professor, Department of Community Medicine, *****Professor, Department of Radiology, LLRM Neducak College, Meerut - 251 001, Uttar Pradesh.*

Corresponding Author: *Dr Vivek Yadav, Junior Resident, Department of Medicine, LLRM Neducak College, Meerut - 251 001, Uttar Pradesh. Tel: 7666487253, E-mail: vivekyaadav@gmail.com.*

patients[8]. HRCT thorax is sensitive in identifying the lung involvement in the early stages of Corona virus disease. It is used for the screening and diagnosis of clinically suspicious COVID-19 patients[9,10]. Though, chest CT scans have been found normal sometimes[11]. This pictorial essay aims to overview the various spectrums of HRCT patterns in COVID-19 pneumonia. Therefore, an important diagnostic tool like HRCT thorax was undertaken to study the correlation of such ongoing long COVID disease symptoms with its severity at initial and acute phase along with various parameters. HRCT severity score was classified as mild with CT severity score (CTSS) < 8, moderate (CTSS 9 - 16) and severe (CTSS 16 - 25) grading on the basis of the scale 0 - 25.

Aims and objectives

1. To evaluate the correlation of HbA1c with HRCT thorax severity score among diabetic and non-diabetic COVID patients.
2. To study the correlation of HRCT severity score with duration of hospital stay of COVID patients with or without diabetes.

Material and Methods

This retrospective case and control single-centered study was done with 162 admitted COVID patients in the tertiary COVID care SVBP hospital during March 2020 to June 2021. The ethical clearance was taken from Ethical committee of the institution LLRM Medical College, Meerut with no./sc-1/2022/7830. The Sample size was calculated as per the formula sample size = $z^2p(1-p)/d^2$, where z is the level of confidence taken as 95%. According to a published study, Prevalence (p) of positive HRCT findings in COVID positive patients was taken as 70% and respectively (1-p) as 30%. Considering absolute precision (d) as 10%, sample size was calculated to 80.67[12,13].

After considering the exclusion criteria, 81 COVID positive diabetic patients were taken as the cases and 81 COVID positive non-diabetic patients as the control. Patients admitted in this hospital during this COVID phase were selected and taken as cases and control. Their demographic, clinical details, laboratory investigations were recorded and radiological imaging was done. Patients were divided into four groups based on HbA1c values. Various blood investigations including ESR, CRP, LFT, KFT, HbA1c and radiological investigations HRCT severity score, CXR PA views were done. The data was collected with the help of questionnaire and was entered in Microsoft Excel Spreadsheets. Statistical tests were applied using SPSS software and chi square value (with confidence interval of 95% and p value < 0.05) was calculated to assess the associations of this study.

Inclusion criteria:

CASE

- Patients who are known cases of diabetes mellitus who had COVID-19 infection
- Age more than >18 yrs

CONTROL

- Patients who are not a known case of diabetes mellitus and had COVID-19 infection
- Age more than >18 yrs

Exclusion criteria:

- Non COVID-19 patients
- ARDS cause other than COVID-19 infection
- Pregnant female
- Active immunological disease
- Evidence of clinical cardiovascular disease (cardiac, cerebral or peripheral vascular disease).
- Immuno-compromised group.
- People with known malignancies.
- Age less than < 18 years and more than > 65 years.

Results

A total of 162 laboratory confirmed COVID-19 patients were enrolled in this study, out of which 81 were diabetic (cases) and 81 were non diabetic (control).

In the cases, i.e., diabetic COVID group 4 cases (4.9%) of the total 81 cases belonged to the age group of (18 - 25 years), 41 cases (50.6%) belonged to the age group of (26 - 50 years) and the remaining 36 cases (44.4%) belonged to the age group of (51 - 65 years).

In the controls, i.e., non diabetic COVID group, 18 cases (22.2%) of total 81 controls belonged to the age group of (18 - 25 years), 35 cases (43.2%) belonged to the age group (26 - 50 years) and 28 cases (34.6%) belonged to the age group (51 - 65 years).

This difference between age groups was significant (with p value of 0.006), i.e., our study had more people of the age above 26 years.

There are 31 (38.3%) females and 50 (61.7%) males in the diabetic group. Non-diabetic group comprised 28 (34.6%) females and 53 (65.4%) males. Males are more than females

but this difference between genders was non-significant (with p value of 0.624).

Table I: HRCT severity score according to HbA1c of the diabetic COVID patients and non-diabetic COVID patients.

	HRCT SEVERITY GRADING								
	Mild score		Modrate score		Severe score		Total		
COVID patients	No.	%	No.	%	No	%	No	%	p-value
Non-diabetic (HbA1c < 5.4%)	25	49.02%	22	43.14%	4	7.84%	51	100%	0.029
Pre-diabetic (HbA1c 5.4 - 6.7%)	20	66.67%	8	26.67%	2	6.67%	30	100%	
Controlled diabetic (HbA1c 6.7 - 8%)	7	35.00%	12	60.00%	1	5.00%	20	100%	
Uncontrolled diabetic (HbA1c > 8%)	23	37.70%	25	40.98%	13	21.31%	61	100%	
Total	75		67		20		162		

Table I shows that, out of 81 diabetic COVID cases 20 patients, i.e., 24.69% patients had controlled diabetes that is HbA1c between 6.7 - 8% and 61 patients (75.31%) had uncontrolled diabetes with HbA1c more than 8%.

Out of 20 controlled diabetic COVID patients, 7 patients (35%) had mild HRCT score, 12 patients (60%) had moderate HRCT score and 1 patient (5%) had severe HRCT score.

Out of 61 uncontrolled diabetic COVID patients, 23 (37.7%) patients had mild HRCT severity score, 25 (40.98%) patients had moderate severity score and 13 (21.31%) patients had severe severity score.

It also shows out of 81 non-diabetic COVID control patients 51 (62.96%) patients were non-diabetic that is HbA1c < 5.4% and 30 patients (37.04%) were pre-diabetic with HbA1c 5.4 - 6.7%.

Out of 51 non-diabetic COVID patients, 25 (49.02%) patients had mild HRCT score, 22 (43.14%) patients had moderate HRCT score and 4 (7.84%) patients had severe HRCT score.

Out of 30 pre-diabetic COVID patients, 20 (66.67%) patients had mild HRCT severity score, 8 (26.67%) patients had moderate severity score and 2 (6.67%) patients had severe severity score.

The association of the HRCT severity score with various HbA1c level patient groups came out as statistically significant with p value of 0.029.

Table II shows that, out of 81 diabetic COVID cases, 30 (37.04%) patients had a Mild HRCT severity score that is less than 8/25, 37 patients (45.67%) had moderate HRCT severity score with score 9 - 16/25, 14 (17.28%) patients had severe HRCT severity scores.

Table II: Shows association of duration of hospital stay with HRCT severity score in diabetic and non-diabetic COVID patients.

Duration of		HRCT Severity grading								
hospital stay in		Mild		Moderate		Severe		Total		
COVID patients		No	%	No	%	No	%	No	%	p-value
Diabetics	1 week	21	80.77%	3	11.54%	2	7.69%	26	100%	0.001
	2 weeks	8	23.53%	23	67.65%	3	8.82%	34	100%	
	>3 weeks	1	4.76%	11	52.38%	9	42.86%	21	100%	
Total		30		37		14		81		
Non-diabetics	1 week	17	80.95%	4	19.05%	0	0.00%	21	100%	0.008
	2 weeks	26	54.17%	19	39.58%	3	6.25%	48	100%	
	>3 weeks	2	16.67%	7	58.33%	3	25.00%	12	100%	
Total	45		30		20		162			

Out of 26 diabetic COVID patients, who had 1 week hospital stay, 21 (80.77%) patients had mild HRCT score, 3 (11.54%) patients had moderate HRCT score, 2 (7.69%) patients had severe HRCT score.

Out of 34 diabetic COVID patients, who had 2 weeks hospital stay, 8 (23.53%) patients had mild HRCT score, 23 (67.65%) patients had moderate HRCT score and 3 (8.82%) patients had severe HRCT score.

Out of 21 diabetic COVID patients, who had more than 3 weeks hospital stay, 1 (4.76%) patient had mild HRCT score, 11 (52.38%) patients had moderate HRCT score and 9 (42.86%) patients had severe HRCT score. There is an increase in duration of hospital stay as per increasing grade of HRCT severity scores in diabetic COVID patients. The association of this HRCT severity score with duration of hospital stay in diabetic COVID group is highly significant with p value of 0.001.

It also shows that out of 81 non-diabetic COVID control patients, 45 (55.56%) patients had a Mild HRCT severity score that is less than 8/25, 30 patients (37.04%) had moderate HRCT severity score with score 9 - 16/25, 6 (7.40%) patients had severe HRCT severity scores.

Out of 21 non-diabetic COVID patients, who had 1 week hospital stay, 17 (80.95%) patients had mild HRCT score, 4 (19.05%) patients had moderate HRCT score, 0 (0.0%) patients had severe HRCT score.

Out of 48 non-diabetic COVID patients who had 2 weeks hospital stay, 26 (54.17%) patients had mild HRCT score, 19 (39.58%) patients had moderate HRCT score and 3 (6.25%) patients had severe HRCT score.

Out of 12 non-diabetic COVID patients, who had more than

3 weeks hospital stay, 2(16.67%) patients had mild HRCT score, 7 (58.33%) patients had moderate HRCT score and 3 (25.0%) patients had severe HRCT score. There is an increase in duration of hospital stay as per increasing grade of HRCT severity scores in non-diabetic COVID patients. The association of this HRCT severity score with duration of hospital stay in non-diabetic COVID group is highly significant with p value of 0.008.

Discussion

Compared with the non-diabetic patients with COVID-19, the diabetic patients with COVID-19 had poorer prognosis and higher mortality. The Study of clinical and imaging characteristics of these patients is helpful to deepen our understanding of the mechanism of critical conditions of the COVID patients with diabetes. It also helps to promote, its early clinical diagnosis and better treatment.

The objective of this case control study was to assess the severity of COVID-19 disease in diabetic patients based on HRCT severity score and HbA1c levels. Most of the patients in diabetic group belonged to the age group of (26 - 50 years), i.e., 41 cases (50.6%) and in non-diabetic group most of the patients also belonged to the age group (26 - 50 years) 35 (43.2%) cases. This difference between age groups in this study was significant (with p value of 0.006), i.e., our study had more people of the age above 26 years. Statsenko *et al* stated with univariate analysis, that the risk of the non-mild COVID-19 was significantly higher ($p < 0.05$) in midlife adults and older adults compared to young adults[14].

In our study, males are more than females in both diabetic and non-diabetic group.

This difference between genders was non-significant (with p value of 0.624). Similar study by Tabassum *et al* 2022 had an increased number of males (85.8%) and lesser number of females (14.2%)[13]. Further studies are needed involving an equal number of females and explore the outcomes in females. Statsenko *et al* showed increased severity in male[14]. Similar findings were seen in our study however more studies needed to be done before coming to any conclusion.

Among 81 patients of the diabetic group, (24.7%) 20 patients were controlled diabetic, i.e., with hbA1c level 6.7 - 8% and (75.3%) 61 cases were uncontrolled diabetic with hbA1c level > 8%.

Among 61 uncontrolled diabetic COVID patients, 13 (21.31%) patients were found to be having severe HRCT findings (i.e., score 16 - 25). Whereas 1 (5%) patient out of 20 controlled COVID diabetic patients had severe HRCT finding.

Among the control group, 2 (6.67%) patients out of 30 pre-diabetic COVID patients and 4 (7.84%) patients out of 51 non-diabetic COVID patients. This shows that poor glycemic control status increases the risk and severity of COVID disease as compared to well controlled diabetics. Thus, it is an imperative to have strict glycemic control during COVID pandaemics.

A similar finding was seen by Rangankar *et al*, who had HRCT severity score of 220 patients, 41 patients had diabetes and 179 patients were non-diabetics. Out of 41 diabetics, severe form of COVID disease on HRCT severity score was found in 12 patients (29.3%), moderate disease in 16 patients (39%) and milder disease in 13 patients (31.7%). COVID disease was severe in 12 diabetic patients (29.3%) as compared to 20 non-diabetic patients (11.7%)[15].

SARS COV-2 has shown to down regulate the ACE2 protein in diabetics and this might be the cause of poor clinical outcome in diabetes patients[16].

In our study, out of 14 diabetic COVID patients, 9 patients having severe HRCT score stayed for more than 3 weeks, 3 (50%) patients with moderate HRCT score stayed for up to 2 weeks, 2 patients with mild CT score stayed for up to 1 week. Whereas out of 6 non-diabetic COVID patients 3 patients having severe HRCT score stayed for more than 3 weeks, 3 (50%) patients having moderate CT score stayed for up to 2 weeks. This difference was significant with p value 0.001 and 0.008 respectively.

A similar study by Saeed *et al*, which showed 3.9% and 4.7% COVID patients with severe and moderate HRCT score respectively, had a hospital stay of more than 16 days[17].

Conclusion

COVID-19 is a lethal viral disease that infects and damages the lung parenchyma. The HRCT severity score of COVID-19 patient increases with poor diabetic control, i.e., HbA1c more than 8%. The association of HRCT severity scores with HbA1c level was very significant. It was found that the poor glycemic control was significantly associated with the disease severity as well as hospital stay. So, strict glycemic control should be observed during such COVID pandemics. The duration of hospital stay of diabetic COVID-19 patient increases with rising HRCT scores and this association was statistically significant. Thus, early HRCT imaging and scoring can prove useful in prognosticating, triaging and determining duration of hospital stay.

Limitations of this study: Small sample size is the limitation of this study. It should be done on larger sample size too.

Conflict of interest: The author has no conflict of interest

to disclose with respect to this study.

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ORIGINAL ARTICLE

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Prevalence and Severity of Non-Alcoholic Fatty Liver Disease in Patients with Chronic Kidney Disease

Shaveta Dahiya, Varun Kulkarni**, Tarana Gupta***, Deepak Jain***, HK Aggarwal*****

Abstract

Introduction: *Recent studies have shown increased prevalence of NAFLD in CKD. The presence and severity of NAFLD has been related to the incidence and stage of CKD independently of traditional CKD risk factors. Further it is postulated that the pathogenic mechanisms causing steatosis and renal injury are common to both, such as insulin resistance, chronic systemic inflammation and dyslipidaemia.*

Objective: *We aimed to evaluate the prevalence of Nonalcoholic Fatty Liver Disease in patients of Chronic Kidney Disease and to determine the severity of Liver Fibrosis in different stages of Chronic Kidney Disease. We also compared efficacy of transient elastography with different fibrosis scores (NFS, FIB-4, APRI) in diagnosing liver fibrosis and estimating its severity.*

Material and methods: *All patients > 18 years with chronic kidney disease, not on renal replacement therapy reporting to Department of Medicine and Nephrology clinic were included into the study and subjected to USG of liver for presence of hepatic steatosis. 100 patients of CKD with evidence of hepatic steatosis on ultrasonography were enrolled into the study who further underwent transient elastography (TE) for determining the severity of liver fibrosis. Fibrosis scores: APRI, NAFLD fibrosis score and FIB-4 index were calculated and specific cut-off values were taken to categorise liver fibrosis into stages.*

Results: *Out of 140 patients of CKD who were screened, 100 patients were found to have evidence of fatty liver, which indicates a prevalence of 71.4%. Use of transient elastography suggested that in patients of stage 3 CKD (n = 24), 3(12.5%) patients had advanced fibrosis, 9 patients had fibrosis F_1-F_2 and 12 patients had no fibrosis. In patients of stage 4 CKD (n = 30), 11(36.6%) patients had advanced fibrosis, 8 patients had fibrosis F_1-F_2 stage and 11 patients had no fibrosis. In stage 5 CKD (n = 46), 33 (71.7%) patients had advanced fibrosis, 8 patients had fibrosis F_1-F_2 and 5 patients had no fibrosis. With increasing stage of CKD, the severity of liver fibrosis increased. Among all non-invasive markers when compared with transient elastography, NAFLD fibrosis score and FIB-4 Index could reliably predict advanced fibrosis in patients with CKD.*

Conclusion: *In our study, we found that NAFLD is significantly associated with CKD with high prevalence in this population. Further we found that advanced fibrosis is significantly more prevalent in advanced CKD stage 5 compared to CKD stages 3 and 4.*

Key words: *CKD, NAFLD, transient elastography, non-invasive scores.*

Introduction

Chronic kidney disease (CKD) is one of the leading causes of chronic diseases globally, with rising incidence and prevalence. The worldwide prevalence of CKD is estimated to be 10.4 - 13.4%[1]. The incidence and prevalence of CKD in India are approximately 0.16% and 0.78%, respectively[2]. Overall CKD mortality has increased by 31.7%, which makes it one of the fastest rising major causes of death[3]. The rising incidence of CKD reflects the rising incidence of obesity, diabetes mellitus (DM), hypertension and metabolic syndrome (MS). Non-alcoholic fatty liver disease (NAFLD) has been defined as accumulation of fat in the liver in the absence of significant alcohol intake, use of medications, or medical conditions that cause fatty liver. The definition also includes the exclusion of other secondary causes of fatty liver, such as presence of hepatitis B and C infection, drug and toxin exposure, surgical procedures, inborn errors of metabolism and total parenteral nutrition. NAFLD has a wide spectrum of disease ranging from 'Non-alcoholic fatty liver' (NAFL), Non-alcoholic steatohepatitis (NASH), NASH-related cirrhosis and NASH-related hepatocellular carcinoma (HCC). NASH is defined as steatosis and inflammation associated with ballooning of hepatocytes, presence of Mallory hyaline bodies, and fibrosis. NASH is usually asymptomatic, but it can present later as cirrhosis of the liver or hepatocellular carcinoma. The worldwide prevalence of NAFLD is estimated to be around 20 - 30% in the general population. Prevalence of NAFLD in Asia is around 5 - 18%. Studies from India suggest a high prevalence of NAFLD varying from 9% to 32%[4]. The prevalence is even higher in high-risk groups like obesity, type 2 DM, hyperlipidaemia and hypothyroidism. In general

**Assistant Professor, **Resident, ***Senior Professor, ****Senior Professor and Head, Department of Medicine, Pt. B.D. Sharma University of Health Sciences, PGIMS, Rohtak - 124 001, Haryana.*

Corresponding Author: *Dr Deepak Jain, Senior Professor, Department of Medicine, Pt. B.D. Sharma University of Health Sciences, PGIMS, Rohtak - 124 001, Haryana. Tel: 9416147887, E-mail: jaindeepakdr@gmail.com.*

the progression of fibrosis in NAFL is believed to be uncommon, but NASH progresses more commonly to fibrosis.

Recent studies have shown the increased prevalence of NAFLD in CKD. The presence and severity of NAFLD has been related to the incidence and stage of CKD independently of traditional CKD risk factors. Conversely, the presence of CKD increases overall mortality in patients with NAFLD compared with the general population. Further supporting a pathogenic link between NAFLD and CKD, NASH-related cirrhosis carries a higher risk of renal failure than other aetiologies of cirrhosis, is an increasing indication for simultaneous liver-kidney transplantation, and is an independent risk factor for kidney graft loss and CVD.

Research has shown that the underlying pathogenic mechanisms causing steatosis and renal injury are common to both, such as insulin resistance, chronic systemic inflammation and dyslipidaemia. Insulin resistance (IR), which is the underlying mechanism of metabolic syndrome has been linked to cause chronic damage to the kidney by causing glomerulosclerosis, podocyte loss and proteinuria and also to the liver in the form of steatosis and fibrosis. Even though only a minority of NAFLD patients progress to significant fibrosis, such patients may develop liver cirrhosis and hepatocellular carcinoma, which have high morbidity and mortality.

The gold standard for the diagnosis and staging of NAFLD is liver biopsy (LB). It is an invasive procedure, with a risk for major complications. Ultrasonography (USG) is the simple, cheap and noninvasive test for assessing fatty infiltration of liver; however, it cannot detect the fibrosis which is the main determinant of progression of liver disease. Transient elastography (TE) has been recently developed for detection of liver stiffness. TE has several advantages, it is non-ionizing, easy to perform, results are operator independent, not relying on subjective interpretation. Based on several studies, variable LSM cut-off values for each stage of fibrosis have been reported, with readings of ≤ 7 kPa as no fibrosis, LSM 7.0 - 12.9 as F_1-F_2 and LSM $\geq$ 13 kPa as advanced fibrosis respectively in NAFLD. Overestimation of fibrosis can occur in cases of hepatitis, cholestasis, liver congestion, obesity, and mass lesions if present in the liver. Several fibrosis scores like NAFLD fibrosis score, FIB-4 index, APRI score, BARD score have been developed to estimate the severity of fibrosis. These non-invasive fibrosis markers have been shown to be associated with liver fibrosis in NAFLD patients. These scores are calculated with routine blood investigations and are a handy tool for bedside assessment of liver fibrosis. NAFLD fibrosis score (NFS) is the most studied score, with external validation in 13 studies, including more than 3,000 patients. It incorporates age, glycaemia, BMI, platelet count, albumin, and AST/ALT ratio and presents great accuracy for advanced fibrosis. A NFS score of < -1.455 indicates F_0-F_2 fibrosis, while a score of > 0.675 is in favour of advanced fibrosis[5].

In this study we aimed to evaluate the prevalence of Nonalcoholic Fatty Liver Disease in patients of Chronic Kidney Disease and to determine the severity of Liver Fibrosis in different stages of Chronic Kidney Disease. We also compared efficacy of transient elastography with different fibrosis scores (NFS, FIB-4,APRI) in diagnosing liver fibrosis and estimating its severity.

Material and methods

Study population

All patients > 18 years with chronic kidney disease, not on Renal replacement therapy reporting to Department of Medicine and Nephrology clinic were included into the study. CKD was defined according to KDIGO 2012 guidelines as eGFR $\leq$ 60 ml/min/1.73 m^2 body surface area for $\geq$ 3 months, irrespective of the cause and patients were classified into stages defined by KDIGO 2012 guideline according to eGFR, calculated by the MDRD eGFR equation[6]. The patients having serological evidence of Hepatitis C, Hepatitis B, history of significant alcohol intake of more than 20 g alcohol per day in men and more than 10 g per day in women, drug treatment causing hepatic steatosis, (e.g., corticosteroids), gastrointestinal bypass surgery, pregnancy, failure of transient elastography (obesity, ascites) and contra-indications for elastography (jaundice, right heart failure, fluid overload, ALT $\geq$ 5 ULN, AST $\geq$ 3 ULN) were excluded.

Data collection

Patients were subjected to USG of liver for presence of hepatic steatosis, performed by an experienced radiologist. Diagnosis of fatty liver was based on USG findings like liver parenchymal brightness, liver to kidney contrast, bright vessel wall and deep beam attenuation. Those patients with evidence of fatty infiltration underwent transient elastography for determining the severity of liver fibrosis. Total 140 patients of chronic kidney disease coming to Nephrology clinic were screened. Out of which 100 patients of CKD with evidence of hepatic steatosis on ultrasonography were enrolled into the study. Body mass index was calculated as weight in kilograms divided by square of height in metre. A total of 100 healthy controls who were age, sex and BMI matched were also taken. Laboratory investigations included complete haemogram, renal and hepatic function tests, serum electrolytes, lipid profile, thyroid profile, fasting blood sugar, HbA_1C levels, urine complete analysis and 24-hour urinary protein.

Transient elastography was conducted on "Fibroscan 502" device with M probe having 3.5 MHz frequency, by a single operator. For every patient, median of minimum 10 successful readings were taken with interquartile range (IQR) of ≤ 30%. Patients were classified as No fibrosis (LSM ≤ 7 kPa), F_1-F_2 (LSM 7.0 - 12.9 kPa) and advanced fibrosis (LSM ≥ 13.0 kPa)[7]. Fibrosis scores: APRI, NAFLD fibrosis score and FIB-4 index were calculated and specific cut-off values were taken to categorise liver fibrosis into stages (Table I and II).

Table I: Non-invasive fibrosis scores and formulae.

Score	Formula
FIB-4	[Age (years) × AST (IU/l)]/[platelet count (109/l) × √ALT (IU/l)]
NFS	− 1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet count (×10^9/l) − 0.66 × albumin (g/dl)
APRI	[{AST (IU/l)/AST (ULN[a])} × 100]/platelet count (10^9/l)

Table II: Fibrosis markers and their cut-off values.

	Advanced Fibrosis	Indeterminate	Advanced fibrosis excluded
NAFLD Fibrosis Score	> 0.675	-1.455 – 0.675	< -1.455
FIB-4 Index	> 3.25	1.45 – 3.25	< 1.45
APRI Score	> 1.5		< 0.5

Statistical analysis

Descriptive statistics were computed as median and mean values. For comparison of quantitative data, Mann Whitney U test and Student's t-test were used. Chi square test with or without Yates correction was used for comparison of qualitative data. Statistical analysis was done using SPSS software v.20 (IBM SPSS Statistics Inc., Chicago, Illinois, USA). Level of significance was set at $p < 0.05$.

Results

Patients of CKD were grouped based upon the eGFR values defined by KDIGO 2012 guidelines as stage 3 (n = 24), stage 4 (n = 30) and stage 5 (n = 46) respectively. Baseline characteristics of the study population are shown in Table III.

Association of Chronic Kidney Disease with Non-alcoholic Fatty Liver Disease

Patients of chronic kidney disease who fit the inclusion and exclusion criteria underwent ultrasonography (n = 140). Out of which, 100 patients were found to have evidence of fatty liver, which indicates a prevalence of 71.4%. A population of age, sex and BMI-matched healthy individuals (n = 100) were taken as controls and hepatic steatosis was seen in 32 individuals on ultrasonography. The prevalence of NAFLD in CKD patients was higher compared to the healthy population and was significantly associated (p value < 0.001) independent of age, sex and BMI.

Table III: Baseline characteristics of study population.

	Stage 3 CKD (n = 24)	Stage 4 CKD (n = 30)	Stage 5 CKD (n = 46)	P value
Age (years)	53 (42, 64)	50 (19, 63)	49 (25, 65)	0.143
Sex (M, F), n	13, 11	16, 14	29, 17	0.640
BMI (Kg/m^2)	24.8 (21.4, 27.3)	23.9 (21.2, 27.4)	24.2 (19.8, 31.1)	0.595
Diabetes, n	12	14	23	0.954
Hb (g/dl)	10.600 (8, 13.1)	10.1 (7.5, 12)	7.8 (5.9, 11.08)	< 0.001
eGFR (ml/min/1.73 m^2 BSA)	35 (31, 47)	21 (11, 29)	7.4 (03, 14)	< 0.001
Blood Urea (mg/dL)	53 (38, 126)	73.5 (48, 156)	207 (21, 302)	< 0.001
Creatinine (mg/dL)	1.8 (1.4, 3.2)	2.8 (2, 5.2)	8 (3.9, 12.9)	< 0.001
Uric acid (mg/dL)	4.5 (2.8, 10.9)	5 (3.8, 11.0)	8.8 (4.6, 14.5)	< 0.001
SGOT (U/L)	37 (17, 64)	42 (27, 66)	44 (24, 73)	0.068
SGPT (U/L)	22.5 (15, 60)	28 (12, 54)	27 (2.9, 66)	0.269
ALP (U/L)	72 (52, 90)	69 (52, 90)	70 (50, 90)	0.868
S. Protein (g/dL)	6.9 (6, 8.8)	7 (5, 7.9)	6 (5, 7)	< 0.001
S. Albumin (g/dL)	3.8 (2, 4.8)	3.8 (2.5, 4)	3 (1, 4)	< 0.001
S. Bilirubin (mg/dL)	0.3 (1.0, 0.630)	0. 600 (0.03, 1.0)	0.600 (0.03, 1.0)	0.209
FBS (mg/dL)	112.5 (80, 236)	97.5 (77, 232)	100 (76, 286)	0.491
HbA1c (%)	6.4 (5.4, 9.5)	6.3 (5, 8.6)	6 (5.3, 10.8)	0.580
Serum TSH (mIU/L)	3.7 (2, 4.5)	3 (2, 4.6)	3.06 (2, 4.7)	0.752
S.Triglyceride (mg/dL)	145 (73, 212)	144.5 (81, 184)	136.5 (83, 209)	0.727
S. Cholesterol (mg/dL)	145.5 (101, 234)	144.5 (101, 256)	142.5 (101, 261)	0.747
HDL(mg/dL)	49 (39, 64)	49 (38, 70)	52 (28, 68)	0.609
LDL(mg/dL)	137.5 (96, 185)	140.5 (104, 180)	127 (59, 188)	0.007
VLDL(mg/dL)	28 (22, 43)	29.5 (16, 42)	29 (17, 43)	0.144

Data expressed as median (Q1,Q3)

These patients were further subjected to transient elastography for determining the severity of liver fibrosis by liver stiffness measurement (LSM). In patients of stage 3 CKD (n = 24), 3 patients had advanced fibrosis, 9 patients had fibrosis F_1-F_2 and 12 patients had no fibrosis. In patients of stage 4 CKD (n = 30), 11 patients had advanced fibrosis, 8 patients had fibrosis F_1-F_2 stage and 11 patients had no fibrosis. In stage 5 CKD (n = 46), 33 patients had advanced fibrosis, 8 patients had fibrosis F_1-F_2 and 5 patients had no fibrosis. With increasing stage of CKD, the severity of liver fibrosis increased. Advanced fibrosis was seen in 12.5% of patients in CKD stage 3, 36.6% in CKD stage 4 and 71.7% in CKD stage 5. From this data, it is clear that NAFLD is significantly associated with CKD and the severity of liver fibrosis increases with increasing stages of CKD (p value 0.04).

Table IV: Association of NAFLD with CKD stages as assessed by USG and transient elastography.

	CKD stages			
	Stage 3 (n = 24)	Stage 4 (n = 30)	Stage 5 (n = 46)	P value
USG grade				
1	17	11	0	< 0.0001
2	7	15	20	
3	0	4	26	
Transient Elastography				
No Fibrosis (n = 28)	12	11	5	0.04
F1-F2 (n = 25)	9	8	8	
Advanced fibrosis (n = 47)	3	11	33	

Table V: Comparison of APRI (AST to platelet ratio) score with fibrosis as assessed by transient elastography.

	Transient Elastography			
	ADF (n = 47)	F1-F2 (n = 25)	NOF (n = 28)	P value
APRI				
No Fibrosis (n = 27)	2	21	4	0.317
Advanced fibrosis (n = 1)	1	0	0	

ADF: Advanced fibrosis. NOF: No fibrosis.

Table VI: Comparison of NAFLD fibrosis score (NFS) with transient elastography.

	Transient Elastography			
	ADF (n = 47)	F1-F2 (n = 25)	NOF (n = 28)	P value
NAFLD Fibrosis score				
No fibrosis (n = 29)	5	21	3	0.03
Advanced fibrosis (n = 44)	38	4	2	

ADF: Advanced fibrosis. NOF: No fibrosis.

Table VII: Comparison of FIB-4 index with transient elastography.

	Transient Elastography			
	ADF (n = 47)	F1-F2 (n = 25)	NOF (n = 28)	P value
FIB-4				
No fibrosis (n = 29)	4	9	18	0.04
Advanced fibrosis (n = 45)	28	10	7	

ADF: Advanced fibrosis. NOF: No fibrosis.

Comparison of Non-Invasive Fibrosis Scores with Transient Elastography

Non-invasive fibrosis scores – APRI, NAFLD fibrosis score and FIB-4 index were calculated and their performance in identifying and grading liver fibrosis were assessed using chi-square test.

APRI score could identify 27 patients as having no fibrosis, out of which 13 patients belonged to CKD stage 3 group, 8 patients belonged to CKD stage 4 group and 6 patients belonged to CKD stage 5 group. Advanced fibrosis could be identified in only 1 patient of CKD. In 72 patients, the grade of fibrosis could not be determined by APRI score. Although APRI score had good sensitivity in identifying patients without liver fibrosis, it could not identify and determine the severity of hepatic fibrosis.

On statistical analysis, it was found out that APRI had a poor performance for identifying and grading the severity of liver fibrosis as compared to TE in patients of CKD (p value 0.317).

NAFLD fibrosis scores revealed 29 patients with no fibrosis. Advanced fibrosis was present in 44 patients of CKD. Advanced fibrosis could be correctly identified in 3 out of 3 patients of CKD stage 3, 9 out of 30 patients in CKD stage 4 and 32 out of 46 patients in CKD stage V respectively. Presence of fibrosis could not be determined in 27 patients. NFS had good sensitivity in identifying patients with advanced fibrosis, however, it could not accurately identify patients with F_1-F_2 grades of fibrosis.

NAFLD fibrosis score was able to independently predict the presence and stage of fibrosis in patients of CKD. The severity of fibrosis increased with increasing stages of CKD. Advanced fibrosis was present in 12.5% of patients in CKD stage 3, 30% in CKD stage 4 and 69% in CKD stage 5.

On further statistical analysis comparing NFS with transient elastography, it was found that NFS was able to identify 38 patients of advanced fibrosis out of 47. NFS was as reliable as transient elastography in identifying patients with advanced fibrosis (p value = 0.03). However, lower grades of liver fibrosis could not be accurately identified.

FIB-4 index scores revealed no fibrosis in 29 patients and advanced fibrosis in 45 patients. 5 patients of CKD stage 3 (n = 24), 7 patients in CKD stage 4 (n = 30) and 33 patients of CKD stage 5 (n = 46) had advanced fibrosis. Grade of fibrosis could not be determined in 26 patients.

FIB-4 index was able to identify the stage of fibrosis in patients of CKD. The severity of fibrosis increased with increasing stages of CKD (p value = 0.03).

On comparing FIB-4 index with transient elastography, statistical analysis showed that the performance of FIB-4 index was comparable to transient elastography in detecting liver fibrosis and quantifying the severity of fibrosis in patients of CKD (p value = 0.04).

Discussion

The pathogenic mechanisms underlying NAFLD

development include increased free fatty acids accumulation, inflammatory cytokines and insulin resistance. NAFLD may alter liver-kidney interactions, including altered renin-angiotensin system activation, antioxidant defense, or lipogenesis, which may contribute to CKD. Another potential link consists of shared susceptibility gene variants between NAFLD and CKD. A recent study conducted by Mantovani *et al*, showed that PNPLA3 polymorphism (I148M variant) was associated with susceptibility to NAFLD development and progression[8]. It was also associated with decreasing eGFR levels and an increased prevalence of CKD in patients with type 2 diabetes. Increases in reactive oxygen species, oxidative stress, and inflammatory response are speculated to be the pathogenic factors involved in NAFLD and CKD. Atherogenic dyslipidaemia (increased small, dense LDL cholesterol, low levels of HDL cholesterol, and high TG levels), which is a common feature of NAFLD, is associated with an increased risk of atherosclerotic diseases including renal endothelial dysfunction and renovascular damage. Recent studies have shown that angiotensin II might also be involved in the progression of NASH as it promotes liver fibrosis and also increases vascular endothelial damage by increased oxidative stress and accelerated atherosclerosis[9]. The contribution toward vascular damage from chronic systemic inflammation could lead to the progression of CKD.

Meta-analysis by Musso *et al* concluded that NAFLD was associated with an increased risk of prevalent and incident CKD[5]. In cross-sectional and longitudinal studies, the severity of NAFLD was positively associated with CKD stages. Study by Mikolasevic *et al*, on 62 CKD patients transient elastography was used to assess liver fibrosis which suggested a high prevalence (85.3%) of NAFLD in these patients[10].

Population based study by Wijarnpreecha *et al* was the first to report the association between the non-invasive fibrosis markers (FIB-4, NFS, APRI, and BARD score) with CKD in adults with ultrasonographic proven NAFLD. High/ intermediate scores of NFS and FIB-4 were associated independently with an increased risk of developing CKD[11]. The findings of this study support the previous studies that showed that FIB-4 is the best marker to distinguish NAFLD patients with CKD compared with other non-invasive fibrosis markers[12].

In a Korean study, NAFLD associated decline in eGFR was higher in patients having proteinuria, low eGFR at baseline, smokers, hypertensives and patients having higher NAFLD fibrosis scores[13].

NAFLD is a frequent comorbidity with CKD with a proportion of patients developing significant liver fibrosis and associated morbidity on long-term. The prevalence is believed to be higher because of higher association of metabolic syndrome with CKD. In our study, we found that NAFLD is significantly associated with CKD with high prevalence in this population. Out of 140 patients of CKD, evidence of NAFLD was found in 100 patients with a prevalence of 71.4 %. The majority of these patients belonged to CKD stage 3 and above. After assessing the severity of liver fibrosis by transient elastography (TE), we found that advanced fibrosis is significantly more prevalent in advanced CKD stage 5 compared to CKD stages 3 and 4. Among all non-invasive markers when compared with transient elastography, NAFLD Fibrosis score and FIB-4 index could reliably predict advanced fibrosis in patients with CKD. Moreover, the severity of advanced fibrosis assessed by NAFLD fibrosis score and FIB-4 index increased with increasing stages of CKD.

To our knowledge, this is one of the few studies demonstrating the association of non-alcoholic fatty liver disease and chronic kidney disease in the Indian population. The prevalence of NAFLD in patients of CKD has also not been studied in the Indian population. Although the gold standard test for diagnosis of NAFLD is liver biopsy, it is an invasive procedure for diagnosis. USG is fairly sensitive for diagnosis of NAFLD, however the findings are subjective. Non-invasive methods like transient elastography and NAFLD scores are easy and rapid methods for predicting advanced fibrosis. For non-invasive assessment, transient elastography has become the gold standard in diseases like chronic hepatitis C and B. Recently, many studies have evaluated transient elastography in NAFLD and found it to be reliable marker of advanced fibrosis.

Recent studies have demonstrated that as the severity of CKD increases, the severity of fibrosis also increases. Patients with CKD stage 5 are more likely to have advanced fibrosis than CKD stage 3 patients. In our study, the severity of liver stiffness was measured by transient elastography. Transient elastography is a highly sensitive screening test to exclude advanced fibrosis in NAFLD. Although optimal cut-off values for staging of fibrosis have varied across studies, a cut-off value of 6.5 kPa can exclude advanced fibrosis with a negative predictive value of 0.91 and LSM values < 12.1 kPa can exclude advanced fibrosis with a negative predictive value of 0.99. A LSM value of ≥ 13 kPa is highly suggestive of advanced fibrosis[14].

Simple serum biomarker panels like APRI, NAFLD fibrosis score and FIB-4 index have shown good accuracy in detecting advanced fibrosis in NAFLD patients. We selected these scores for our study because they have been widely validated in NAFLD patients and are relatively easy to perform. In a meta-analysis of 59 studies involving over 12,558 patients of NAFLD, the AUROC values were

found to be 0.77, 0.84 and 0.84 for APRI, NFS and FIB-4 index respectively for detecting advanced fibrosis. The NPV of these scores for excluding advanced fibrosis was high (89 - 93%). However, the PPV values were modest 55 - 67% potentially leading to false positive results. Approximately 30% cases may show indeterminate results[15]. More complicated biomarkers like Enhanced Liver Fibrosis (ELF) score, hepascore are more accurate, but are costly and have less utility in resource limited settings.

In our study, APRI score could detect only one patient with advanced fibrosis and therefore had a poor performance as compared to transient elastography for detection of fibrosis.

FIB-4 score is considered the most accurate score for diagnosing advanced fibrosis in NAFLD. In our study, the performance of FIB-4 Index in detection of advanced fibrosis was comparable to transient elastography. Studies have shown that patients with a higher value of FIB-4 index had greater deterioration of eGFR over a follow-up of 3 years[12].

NAFLD fibrosis score (NFS) is the most studied score, with external validation in 13 studies, including more than 3,000 patients[5,16,17]. In our study, presence of advanced fibrosis could be detected in 44 patients of CKD by NFS. The severity of fibrosis increased with increasing stages of CKD. Performance of NFS was comparable to transient elastography for diagnosing advanced fibrosis in CKD patients.

Limitations of the study

Our study was a cross-sectional study design which does not allow the establishment of a causal relationship between NAFLD and CKD. Second, the diagnosis of NAFLD was not confirmed by liver biopsy. It is known that only liver biopsy can certainly assess the severity of damage and the prognosis. However, liver biopsy would be impractical to perform in routine health examinations. Third, sample size of the study was only 100. Our study population had 49% of diabetes patients. As diabetes patients frequently have higher BMI and more prevalence of fatty liver disease, this could have been a confounding factor in our study.

Conclusion

This study suggests that NAFLD is highly prevalent in CKD patients. The severity of liver fibrosis is negatively associated with the kidney function. Whether interventions to interfere with frequent connection of CKD with obesity, type 2 diabetes, dyslipidaemia would reduce the risk of developing NAFLD is not known. Although current guidelines do not recommend screening for NAFLD in CKD patients, individuals with CKD should be screened for NAFLD even in the absence of classical risk factors. Transient elastography and non-invasive fibrosis markers (NFS and FIB-4 index) are reliable methods for screening advanced fibrosis in CKD patients. However, fibrosis staging could not be determined accurately in up to 30% of patients of NAFLD using fibrosis scores. A concurrent use of fibrosis scores and transient elastography can be used to improve the accuracy. Early diagnosis of NAFLD can help in aggressive management to retard the progression to cirrhosis and decrease the morbidity and mortality associated with chronic kidney disease.

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ORIGINAL ARTICLE

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Evaluation of Risk Factors for Cardiovascular Disease in Patients of Chronic Kidney Disease

HK Aggarwal, Deepak Jain**, Sakshi Mittal***, Shaveta Dahiya*****

Abstract

Introduction: *We sought to determine the influence of risk factors of chronic kidney disease (CKD) on cardiovascular disease. We studied the risk factors for cardiovascular disease (lipid profile, HbA1C, coronary artery calcium score) in predialysis patients of CKD.*

Material and methods: *100 patients of CKD and 30 healthy controls with age and sex matched were enrolled. CKD patients were further divided into stages according to eGFR. Patients with history of CVD, history of predisposing factors to dyslipidaemia were excluded from the study. The following biochemical parameters were done in all patients-haemoglobin (g/dL), blood urea (mg/dL), serum creatinine (mg/dL), serum calcium corrected for albumin (mg/dL), serum phosphorus (mg/dL), iPTH, serum uric acid (mg/dL), eGFR, urine examination, serum albumin (g/dL), ECG, ultrasonography bilateral kidneys, CRP, homocysteine, fasting blood sugar, HbA1C, lipid profile, etc. Coronary artery calcification was detected with computed tomography.*

Results: *In present study, the most common cause of CKD was hypertension (45%) followed by type-2 diabetes mellitus (30%). Systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in CKD patients were 142.3 ± 15.99 and 85.04 ± 7.04, respectively and it was significantly increased with a decline in eGFR (p value = < 0.05). The triglycerides, cholesterol and LDL lipids were significantly increased in CKD patients than control group and these were significantly increased with increasing in CKD stages (p value = < 0.0001, 0.018, < .0001 respectively) and HDL lipid was significantly low with an increasing in CKD stages. HbA1C (%) in CKD patients was 5.77 ± 1.21, and in control group was 4.99 ± 1.42, (p value = 0.009) but this was not statistically significant in different stages of CKD. CAC score was significantly present in CKD patients than in the control group (p value = < .0001). In CKD patients, out of 100 patients, calcification was present in 65 patients, while in the control group, only minimal calcification was present in 6 patients. The mean ± SD of CAC score in stage 5 was 110 ± 79.48, which was significantly high as compared to stage 4 (67.42 ± 61.41) and stage 3 (22.91 ± 39.44) (p value = < .0001), while in control group, the mean ± SD of CAC score was 1.27 ± 2.79. On performing multivariate regression analysis, With the increase in triglyceride (mg/dL), serum homocysteine (µmol/L), CRP (mg/dL), i-PTH (pg/mL), eGFR (mL/min/1.73 m^2) by 1-unit, CAC score significantly increased by 0.477, 2.802, 12.255, 0.426, 1.412 units, respectively.*

Conclusion: *Risk factors of CVD are highly prevalent in CKD patients. Cardiac calcification should be considered as a marker of CVD risk in CKD patients and it improves risk prediction for CVD. Various traditional and non-traditional risk factors such as increased CRP, iPTH, homocysteine, anaemia, hyperphosphataemia, dyslipidaemia, insulin resistance etc., accelerate the rate of cardiac calcification. Present study showed that CAC score, lipid profile and CVD are reliable markers for screening CVD in CKD patients.*

Key words: *CKD, CVD, HbA1C, hyperphosphataemia, dyslipidaemia.*

Introduction

Chronic kidney disease (CKD) is one of the leading causes of non-communicable diseases globally, with rising incidence and prevalence. Patients with CKD are a considerable social and economic burden, both directly in terms of resource use and indirectly in lost productivity and reduced quality of life. The worldwide increase of CKD is mainly driven by the rise in the prevalence of diabetes mellitus, hypertension, obesity, and aging[1]. According to the 2015 Global Burden of Disease Study, CKD was the 12th leading cause of death, accounting for 1.1 million fatalities worldwide, and the 17th leading cause of disability. Overall, CKD mortality has increased by 31.7%, making it one of the fastest rising significant causes of death[2]. The global all-age mortality rate due to CKD is increased by 41.5% between 1990 and 2017. CKD also became the 19th leading cause of years of life lost in 2013, compared with being the 36th leading cause in 1990. Subsequent GBD (Global burden of disease) reports indicate that the CKD will become the fifth highest cause of years of life lost globally by 2040[3].

CKD patients suffer from many complications, such as hypertension, cardiovascular diseases, anaemia, metabolic acidosis, altered immune response, mineral and bone disturbances, and neurological complications. Among these complications, cardiovascular complications are

**Senior Professor and Head, **Senior Professor, ***Resident, ****Assistant Professor, Department of Medicine, Pt. B.D. Sharma University of Health Sciences, PGIMS, Rohtak - 124 001, Haryana.*

Corresponding Author: Dr Deepak Jain, Senior Professor, Department of Medicine, Pt. B.D. Sharma University of Health Sciences, PGIMS, Rohtak - 124 001, Haryana. Tel: 9416147887, E-mail: jaindeepakdr@gmail.com.

widespread and the most common cause of death. CVD is approximately three times more frequent in patients with CKD than in other known cardiovascular risk groups. Similar cardiovascular mortality is approximately 10-fold more frequent in CKD patients than in the age- and sex-matched segments of the non-renal population[4]. Cardiovascular mortality has been estimated to be around 9% per year. Patients with CKD are predisposed to CVD in various forms, including coronary artery disease, atrial or ventricular arrhythmias, myocardial infarction, stroke, congestive heart failure, or peripheral vascular disease[5].

The presence of cardiac calcification is an appropriate marker and strong predictor of cardiovascular disease and all cause mortality in CKD patients and correlates directly with the amount of coronary plaque and cardiac calcification in highly advanced CKD patients. Also, the degree of cardiac calcification can be prognosticating by coronary artery calcium score (CACS). It may affect the arterial media, atherosclerotic plaques, myocardium, and heart valve. Pathomorphologically, in the general population, calcification involves the intimal layer more than the medial layer i.e., atherosclerosis, while in CKD patients calcification involves the medial layer more than the intimal layer, i.e., arteriosclerosis.

Numerous risk factors are accountable for cardiovascular disease and CAC in CKD patients, which are predominantly classified in traditional and non-traditional groups. Traditional risk factors include age, gender, race, menopause, family history, dyslipidaemia, hypertension, DM; and non-traditional risk factors include anaemia, hypoalbuminaemia, abnormal mineral metabolism, inflammation, oxidative stress, hyperhomocysteinaemia, uric acid, etc., in the development of cardiovascular disease in CKD patients. Cardiovascular mortality and morbidity are much higher in CKD patients and cannot be fully explained on the basis of traditional risk factors only. Hence more interest has focussed on the role of other non-traditional risk factors.

This study was conducted to evaluate the ideal modality of assessment of CVD in CKD patients and importance of CAC in assessment of the CVD in CKD patients. This study also assesses the rate and potential risk factors of CAC progression in CKD patients. The available data indicates the burden of CVDs and CAC in CKD patients on dialysis, but there is scarcity of data in patients who are not yet on dialysis. Hence, the present study was planned to evaluate the risk factors of CVD in CKD patients, especially CAC.

Materials and method

The present study was a single centre, cross-sectional, observational study. 100 patients of CKD of age 18 - 60 years were enrolled, who were not yet on haemodialysis and classified into stages (stage 3 - 5) based on eGFR, calculated by MDRD equation. Chronic kidney disease was defined according to KDIGO 2012 Guidelines as eGFR < 60 mL/min/1.73 m^2 body surface area for more than three months, irrespective of the cause[6]. 30 patients were also taken, age and sex-matched, who had no evidence of structural kidney disease and no radiological and biochemical evidence of renal insufficiency as control.

Patients with aged less than 18 years and greater than 60 years of age, history of CVD, history of predisposing factors to dyslipidemia like liver disease and hypothyroidism, history of drug treatment causing altered lipid levels like statins, alpha-, and beta-blockers, history of inflammatory disease, autoimmune disorders, neoplasms, and disseminated intravascular coagulation (DIC), history of genetic lipid disorders, pregnancy or known psychiatric disorder, were excluded from the study.

After screening the patients for inclusion and exclusion criteria, a detailed history was taken, and a thorough physical examination was performed. Various anthropometric parameters like height and weight were measured in all patients. Waist hip ratio (WHR) was calculated from standard nomograms. The following biochemical parameters were done in all patients-haemoglobin (g/dL), total leukocyte count, blood urea (mg/dL), serum creatinine (mg/dL), serum electrolytes (mEq/L): Sodium and potassium, serum calcium corrected for albumin (mg/dL), serum phosphorus (mg/dL), iPTH, serum uric acid (mg/dL), eGFR, urine complete examination, serum albumin (g/dL), ECG, ultrasonography bilateral kidneys, CRP, homocysteine, fasting blood sugar, HbA1C, lipid profile. HbA1c was measured using high-performance liquid chromatography (HPLC).

Fasting lipid profiles were obtained for each patient at the time of enrollment. The patient's serum was used to estimated total cholesterol, HDL cholesterol, and triglycerides. LDL cholesterol was then calculated by using the Friedewald equation: LDL cholesterol (mg/dL) = total cholesterol – HDL cholesterol – (triglycerides/5). Patients underwent non-contrast, high resolution computed tomography scans for CAC score. HRCT cine acquisition was collected contiguous axial slices from the tracheal carina to the inferior margin of the heart. All areas of calcification with a minimum density of 130 Hounsfield Units within the borders of the coronary arteries were computed. Images were recorded during breath-holding sessions. The acquired images were reviewed on a dedicated workstation, and the Agatston method was applied for calculating a calcium score which incorporates the density of calcification, multiplying the calcification volume by a weighted density co- efficient. A CACS was calculated individually for the left main, left circumflex, left anterior descending, posterior

descending, and right coronary arteries. The score was then summed to calculate the total score.

CAC score	Atherosclerotic plaque burden	Probability of significant CAD	Implication for cardiovascular risk
0	No identifiable plaque	Very low, <5%	Very low
1 - 10	Minimal identifiable plaque burden	Very unlikely, <10%	Low
11 - 100	Definite, at least mild plaque burden	Mild or minimal coronary stenosis is likely	Moderate
101 - 400	Undeniable, at least reasonable plaque burden	Non-obstructive CAD likely	Moderately high
> 400	Extensive plaque burden	High likelihood (> 90%) of at least one significant coronary stenosis	High

Statistical analysis

The quantitative data were presented as the means ± SD. The following statistical tests were applied for the results: The quantitative variables' comparison was analysed using an Independent t-test (for two groups) and ANOVA (for more than two groups). The qualitative variables' comparison was analysed using the Chi-Square test. Pearson correlation co-efficient was used for correlation of quantitative parameters. Univariate and multivariate linear regression was used to determine the coronary calcium score factors. A p-value of less than 0.05 was considered statistically significant for statistical significance.

Results

Of the 130 patients enrolled in this study, 100 patients had CKD and 30 were age and sex matched control. Out of 100 CKD patients, 27 patients were in stage 3, 48 were in stage 4 and 25 patients were present in stage 5 CKD. The baseline clinical and biochemical data of control group and CKD patients, and in different stages of CKD are reported in Table I and II, respectively.

In present study, the mean ± SD age (years) in control was 58.4 ± 8.14 and in cases was 54.76 ± 12.06, (p value = 0.061) and the mean ± SD age (years) in CKD stage 5 was 55.6 ± 13.09, which was slightly higher than CKD stage 4 (55.77 ± 11.06) and CKD stage 3 (52.19 ± 12.85), (p value = 0.434). In this study, total 64 males and 66 females were included, from which 13 females and 17 males were taken in the control group and 53 females and 47 males were taken as cases, (p value = 0.353). The common aetiology of renal disease in study population were hypertension (45%), diabetes mellitus (30%), chronic glomerulonephritis (9%), autosomal polycystic kidney disease (10%), obstructive uropathy (3%), pyelonephritis. Diabetic patients were comparable in both group (30%) and hypertension were significantly high in CKD patients than control group (p value < 0.001).

Table I: Comparison of biochemical investigations between control and CKD patients.

Biochemical investigations	Control (n = 30)	CKD (n = 100)	P value
Systolic blood pressure (mmHg)	126.27 ± 17.22	142.3 ± 15.99	< .0001
Diastolic blood pressure (mmHg)	80.53 ± 7.52	85.04 ± 7.04	0.003*
Waist hip ratio	0.86 ± 0.05	0.86 ± 0.05	0.672
Diabetes mellitus type 2	9 (30%)	30 (30%)	1§
Blood urea (mg/dL)	22.57 ± 5.18	100.14 ± 24.92	< .0001*
Serum creatinine (mg/dL)	0.73 ± 0.09	3.04 ± 1.05	< .0001*
Sodium (mEq/L)	140 ± 0	139.15 ± 3.2	0.009*
Potassium (mEq/L)	4.11 ± 0.07	4.23 ± 0.51	0.03*
Serum uric acid (mg/dL)	4.38 ± 0.67	6.19 ± 2.12	< .0001*
eGFR (mL/min/1.73 m^2)	98.8 ± 6.96	23.1 ± 9.17	< .0001*
Serum albumin (g/dL)	4.18 ± 0.31	3.21 ± 0.46	< .0001*
Serum calcium corrected for albumin (mg/dL)	9.3 ± 0.73	8.61 ± 0.61	< .0001*
Serum phosphorus (mg/dL)	3.64 ± 0.59	5.36 ± 1.55	< .0001*
i-PTH (pg/mL)	44.53 ± 19.3	197.95 ± 38.52	< .0001*
Product of serum calcium corrected and phosphorus	33.87 ± 6.43	45.56 ± 11.42	< .0001*
Triglyceride (mg/dl)	134.47 ± 26.48	149.67 ± 31.37	0.017*
Cholesterol(mg/dl)	191.83 ± 37.1	213.27 ± 21.54	0.005*
HDL (mg/dl)	46.57 ± 5.53	39.41 ± 5.35	< .0001*
LDL(mg/dl)	134.7 ± 23.12	151.83 ± 32.13	0.008*
Fasting blood sugar (mg/dl)	105.07 ± 13.64	113.14 ± 27.55	0.032*
HbA1C (%)	4.99 ± 1.42	5.77 ± 1.21	0.009*
Serum homocysteine (µmol/L)	7.23 ± 2.36	26.56 ± 7.23	< .0001*
C-reactive protein (mg/dL)	0.39 ± 0.22	5.67 ± 2.76	< .0001*

* *Independent t-test*

Table II: Baseline characteristics of CKD patients in different stages.

Characteristics	Stage 3 (n = 27)	Stage 4 (n = 48)	Stage 5 (n = 25)	P value
Age	52.19 ± 12.85	55.77 ± 11.06	55.6 ± 13.09	0.434‡
Female	13 (48.15%)	26 (54.17%)	14 (56%)	0.83**
Male	14 (51.85%)	22 (45.83%)	11 (44%)	0.83**
Waist hip ratio	0.86 ± 0.05	0.86 ± 0.05	0.84 ± 0.05	0.441‡
Systolic blood pressure (mmHg)	128.67 ± 13.64	146.92 ± 12.11	148.16 ± 16.7	< .0001‡
Diastolic blood pressure (mmHg)	80.59 ± 7.82	86.83 ± 5.23	86.4 ± 7.33	0.0004‡
Diabetes Mellitus	10 (37.04%)	11 (22.92%)	9 (36%)	0.331§
Haemoglobin (g/dL)	11.93 ± 1.04	10.23 ± 0.82	8.14 ± 0.67	< .0001‡
Blood urea (mg/dL)	84.95 ± 20.24	98.94 ± 21.63	118.86 ± 23.97	< .0001‡
Serum creatinine (mg/dL)	2.03 ± 0.44	2.88 ± 0.6	4.43 ± 0.63	< .0001‡
Serum sodium (mEq/L)	139.33 ± 2.9	138.29 ± 3.43	140.6 ± 2.52	0.012‡

Serum potassium (mEq/L)	4.29 ± 0.55	4.14 ± 0.48	4.34 ± 0.53	0.244‡
Uric acid (mg/dL)	6.04 ± 2.66	6.16 ± 2.04	6.41 ± 1.66	0.817‡
eGFR (mL/min/1.73 m^2)	35.26 ± 5.62	21.6 ± 4.03	12.84 ± 0.9	< .0001‡
Serum albumin (g/dL)	3.57 ± 0.43	3.15 ± 0.41	2.93 ± 0.35	< .0001‡
Serum calcium corrected for albumin (mg/dL)	9.3 ± 0.41	8.58 ± 0.32	7.91 ± 0.36	< .0001‡
Serum phosphorous (mg/dL)	3.84 ± 0.93	5.31 ± 0.87	7.09 ± 1.31	< .0001‡
iPTH (pg/mL)	179.3 ± 28.39	197.12 ± 34.23	219.68 ± 45.49	0.0005‡
Product of serum calcium corrected and phosphorus	35.73 ± 8.78	45.62 ± 7.84	56.05 ± 10.53	< .0001‡
Triglyceride (mg/dL)	131.74 ± 22.47	143.6 ± 23.96	180.68 ± 30.87	< .0001‡
Total cholesterol (mg/dL)	205.07 ± 23.64	213.4 ± 17.75	221.88 ± 23.22	0.018‡
LDL (mg/dL)	136.22 ± 30	144.54 ± 22.97	182.68 ± 29.63	< .0001‡
HDL (mg/dL)	44.52 ± 4.3	39.6 ± 3.36	33.52 ± 3.19	< .0001‡
CRP (mg/dL)	3.11 ± 1.32	5.53 ± 1.86	8.69 ± 2.4	< .0001‡
Homocysteine (µmol/L)	19.7 ± 4.95	26.27 ± 4.56	34.52 ± 5.41	< .0001‡
HbA1C (%)	5.48 ± 1.17	5.78 ± 1.09	6.08 ± 1.45	0.208‡

‡ANOVA

Mean values of systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in CKD patients were 142.3 ± 15.99 and 85.04 ± 7.04, respectively and in control group were 126.27 ± 17.22 and 80.53 ± 7.52, respectively (p value = < .0001, 0.003). In present study, we observed that BP was significantly increased with a decline in eGFR (p value = < 0.05). Mean ± SD of waist-hip ratio in control was 0.86 ± 0.05 and in CKD patients was 0.86 ± 0.05, (p value = 0.672).

In the present study, haemoglobin, blood urea, serum creatinine, eGFR, serum albumin, uric acid, calcium, phosphorus and their products, iPTH, serum sodium and potassium were significantly high in cases than the control group, (p value = < .0001). It also found significant differences in haemoglobin, blood urea, serum creatinine, eGFR, serum albumin, serum calcium, serum phosphorus, serum phosphorous and corrected serum calcium product and iPTH between different stages of CKD, (p value = < 0.05). The mean ± SD of HDL (mg/dL) in control was 46.57 ± 5.53 and in CKD was 39.41 ± 5.35 (p value = < 0.0001)). Mean ± SD values of triglyceride (mg/dL), cholesterol (mg/dL), LDL (mg/dL) in CKD patients were 149.67 ± 31.37, 213.27 ± 21.54, 151.83 ± 32.13, respectively and in control group were 134.47 ± 26.48, 191.83 ± 37.1, 134.7 ± 23.12, respectively (p value = 0.01, 0.005, 0.008 respectively). The HDL and non- HDL lipids were significantly different in different stages of CKD, (p value = < 0.0001). The mean ± SD of fasting blood sugar (mg/dL) and HbA1C (%) in CKD patients were 113.14 ± 27.55 and 5.77 ± 1.21, respectively and in control group were 105.07 ± 13.64 and 4.99 ± 1.42, respectively (p value = 0.032, 0.009 respectively). But HbA1C and fasting blood sugar were statistically non significant in different stages of CKD, (p value = 0.612, 0.208). There were significant difference in serum homocysteine (µmol/L) and CRP (mg/dL) levels in cases and controls (p value = <.05). The mean ± SD of serum homocysteine (µmol/L) and CRP (mg/dL) in cases were 26.56 ± 7.23, 5.67 ± 2.76, respectively and in control group were 7.23 ± 2.36, 0.39 ± 0.22, respectively (p value = < .0001)). It also showed significant difference in homocysteine and CRP in different stages of CKD (p value < .0001).

Baseline CAC is shown in Table III (A and B). In present study, CAC score was significantly present in CKD patients than in the control group (p value = < .0001). In CKD patients, out of 100 patients calcification was present in 65 patients. Out of 100 patients, minimal calcification was present in 2 patients, mild calcification was present in 37 patients and moderate calcification was present in 26 patients. We didn't find severe calcification (> 400) in any group. In the control group, only minimal calcification was present in 6 patients. It also found that the severity of calcification was increased with increasing CKD stages (p value = 0.005). The mean ± SD of CAC score in stage 5 was 110 ± 79.48, which was significantly high as compared to stage 4 (67.42 ± 61.41) and stage 3 (22.91 ± 39.44) (p value = < .0001).

Table III (a): Comparison of coronary calcium score between control and CKD patients.

CAC score	Control (n = 30)	CKD (n = 100)	Total	P value
CAC score 0	24 (80%)	35 (35%)	59 (45.38%)	< .0001†
CAC score 1 - 10	6 (20%)	2 (2%)	8 (6.15%)	
CAC score 11 - 100	0 (0%)	37 (37%)	37 (28.46%)	
CAC score 101 - 400	0 (0%)	26 (26%)	26 (20%)	
CAC score > 400	0 (0%)	0 (0%)	0 (0%)	
Mean ± SD	1.27 ± 2.79	66.05 ± 68.75	51.1 ± 66.18	< .0001*

**Independent t test, †Fisher's exact test.*

Table III (b): Comparison of CAC score between stages of CKD.

CAC Score	Stage 3 (n = 27)	Stage 4 (n = 48)	Stage 5 (n = 25)	Total	P value
CAC score 0	15 (55.56%)	15 (31.25%)	5 (20%)	35 (35%)	0.005†
CAC Score 1 - 10	2 (7.41%)	0 (0%)	0 (0%)	2 (2%)	
CAC Score 11 - 100	7 (25.93%)	22 (45.83%)	8 (32%)	37 (37%)	
CAC Score 101 - 400	3 (11.11%)	11 (22.92%)	12 (48%)	26 (26%)	
CAC Score > 400	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Mean ± SD	22.91 ± 39.44	67.42 ± 61.41	110 ± 79.48	66.05 ± 68.75	< .0001‡

†Fisher's exact test, ‡ANOVA.

In the present study we found, severity of CAC was increased with age but it was statistically non-significant, (p value - 0.931). CAC was significantly increased with decrease in eGFR. In particular, with 0 CAC score, the mean

eGFR was 26.63 ± 9.83 and with CAC score 1 - 100, the mean eGFR was 22.38 ± 8.26 and eGFR was 19.42 ± 8.12 with > 100 CAC score, (p value - 0.007). Severity of calcification was also increased with increasing in severity of CRP, hypoalbuminaemia, hypocalcaemia, hyperphosphataemia, hyperparathyroidism, dyslipidaemia. It was also affected by hyperhomocysteinaemia and insulin resistance, (p value - < 0.0001) (Table IV).

Table IV: Association of various parameters with coronary calcium score in CKD.

Mean ± SD	0 (n = 35)	1 to 100 (n = 39)	> 100 (n = 26)	P value
Age (years)	54.46 ± 13.42	54.51 ± 12.6	55.54 ± 9.44	0.931‡
eGFR (mL/min/1.73 m^2)	26.63 ± 9.83	22.38 ± 8.26	19.42 ± 8.12	0.007‡
Serum albumin (g/dL)	3.41 ± 0.53	3.19 ± 0.39	2.95 ± 0.33	0.0004‡
Serum calcium corrected for albumin (mg/dL)	8.72 ± 0.67	8.68 ± 0.54	8.35 ± 0.6	0.039‡
Serum phosphorus(mg/dL)	3.95 ± 0.95	5.67 ± 0.86	6.79 ± 1.43	< .0001‡
i-PTH (pg/mL)	165.29 ± 10.25	195 ± 25.56	246.35 ± 28.23	< .0001‡
Product of serum calcium corrected and phosphorus	33.95 ± 6.5	48.9 ± 5.31	56.16 ± 9.66	< .0001‡
Triglyceride (mg/dL)	122.54 ± 13.54	146.26 ± 16.36	191.31 ± 19.76	< .0001‡
Cholesterol (mg/dL)	194.2 ± 11.09	216.28 ± 14.39	234.42 ± 19.16	< .0001‡
HDL (mg/dL)	43.14 ± 5.36	38.79 ± 3.85	35.31 ± 3.74	< .0001‡
LDL (mg/dL)	124.34 ± 13.01	151 ± 23.29	190.08 ± 21.28	< .0001‡
Serum homocysteine (µmol/L)	19.97 ± 4.41	26.79 ± 4.09	35.08 ± 4.34	< .0001‡
C-reactive protein (mg/dL)	3.11 ± 1.11	5.74 ± 1.43	8.98 ± 2.17	< .0001‡
HbA1C (%)	4.89 ± 1.29	6.17 ± 0.69	6.37 ± 1.07	< .0001‡

‡ANOVA

In the present study, significant positive correlation of CAC score was found with triglyceride (mg/dL), cholesterol (mg/dL), LDL (mg/dL), HbA1C (%), serum homocysteine (µmol/L), CRP (mg/dL), i-PTH (pg/mL), serum phosphorus(mg/dL), product of serum calcium corrected and phosphorus with correlation coefficient of 0.871, 0.679, 0.787, 0.48, 0.87, 0.89, 0.868, 0.77, 0.791, respectively. There was also significant negative correlation of CAC score with HDL (mg/dL), serum calcium corrected for albumin (mg/dL), eGFR (mL/min/1.73 m^2), serum albumin (g/dL) with correlation coefficient of -0.628, -0.313, -0.427, -0.406, respectively. No correlation was seen between CAC score with age (years) with correlation coefficient of 0.024.

Effect of insulin resistance on CAC is shown in Table VI. In present study, CAC was comparable between diabetics and non-diabetics (p value = 0.642).

The mean ± SD of CAC score in diabetic patients was 73.07 ± 80.31, slightly higher than non-diabetic patients (63.04 ± 63.57), but it was not statistically significant (p value = 0.507).

Table V: Correlation of coronary calcium score with various parameters in CKD.

Variables	Coronary Calcium Score	
	Correlation co-efficient	P value
Triglyceride (mg/dL)	0.871	< .0001
Cholesterol (mg/dL)	0.679	< .0001
HDL (mg/dL)	-0.628	< .0001
LDL (mg/dL)	0.787	< .0001
HbA1C (%)	0.48	< .0001
Serum homocysteine (µmol/L)	0.87	< .0001
C-reactive protein (mg/dL)	0.89	< .0001
i-PTH (pg/mL)	0.868	< .0001
Serum calcium corrected for albumin (mg/dL)	-0.313	0.002
Serum phosphorus (mg/dL)	0.77	< .0001
Product of serum calcium corrected and phosphorus	0.791	< .0001
Age (years)	0.024	0.813
eGFR (mL/min/1.73 m^2)	-0.427	< .0001
Serum albumin (g/dL)	-0.406	< .0001

Pearson correlation coefficient

Table VI: Comparison of coronary calcium score between diabetic and non-diabetic in CKD.

Coronary calcium score	Diabetic CKD (n = 30)	Non-diabetic CKD (n = 70)	P value
Evidence of CAD: 0 calcium score	11 (36.67%)	24 (34.29%)	0.642†
No Minimal CAD: 1 - 10	1 (3.33%)	1 (1.43%)	
Mild CAD: 11 - 100	9 (30%)	28 (40%)	
Moderate CAD: 101 - 400	9 (30%)	17 (24.29%)	
Mean ± SD	73.07 ± 80.31	63.04 ± 63.57	0.507*

**Independent t-test, †Fisher's exact test.*

Table VII: Multivariate linear regression to determine the factors affecting coronary calcium score.

Variables	Beta co-efficient	Standard error	P value	Lower bound (95%)	Upper bound (95%)
Triglyceride (mg/dL)	0.477	0.203	0.021	0.073	0.881
Cholesterol (mg/dL)	0.010	0.165	0.952	-0.318	0.338
HDL (mg/dL)	0.161	0.783	0.838	-1.396	1.718
LDL (mg/dL)	-0.196	0.162	0.229	-0.517	0.125
HbA1C (%)	1.362	2.282	0.552	-3.174	5.898
Serum homocysteine (µmol/L)	2.802	1.164	0.018	0.487	5.116
C-reactive protein (mg/dL)	12.255	3.264	0.0003	5.767	18.744
i-PTH (pg/mL)	0.426	0.122	0.001	0.183	0.668
Serum calcium corrected for albumin (mg/dL)	6.707	14.040	0.634	-21.203	34.617
Serum phosphorus (mg/dL)	-7.410	21.030	0.725	-49.217	34.397
Product of serum calcium corrected and phosphorus	0.767	2.452	0.755	-4.107	5.641
eGFR (mL/min/1.73 m^2)	1.412	0.479	0.004	0.459	2.364
Serum albumin (g/dL)	-4.211	6.309	0.506	-16.752	8.330

On multivariate regression analysis, triglyceride, serum homocysteine, CRP, i-PTH, eGFR were significant independent factors affecting CAC score after adjusting for confounding factors. With the increase in triglyceride (mg/dL), serum homocysteine (µmol/L), CRP (mg/dL), i-PTH (pg/mL), eGFR (mL/min/1.73 m^2) by 1-unit, CAC score significantly increased by 0.477, 2.802, 12.255, 0.426, 1.412 units, respectively (Table VII).

Discussion

CKD is a global health burden with a high economic cost to health system and is an independent risk factor for CVD. All stages of CKD are associated with increased risks of cardiovascular morbidity, mortality and decreased quality of life. So, assessing the risk of CVD in CKD patients is essential. The risk of CVD can be predicted by CAC score, carotid intima-media thickness, ankle-brachial index, brachial flow-mediated dilation and high-sensitivity CRP. Many studies showed that CAC score according to Agatston Classification, is the most appropriate method to classify persons into CVD risk categories[7,8]. So, in present research, CAC score was taken as an indicator of CVD progression. It is a noninvasive marker of subclinical atherosclerosis and has been an independent predictor of cardiovascular events and also endorsed by the American Diabetes Association[9].

In present research, CAC was significantly high in CKD patients than control group. Out of 100 patients with CKD, evidence of CAC was found in 65% and in control group calcification was present in 20 % of people i.e., 6 individuals. This is not fully explained by Framingham's traditional risk factors, which are also present in the general population. Some non-traditional risk factors specifically present in CKD patients, also contributed to this. Elraoof *et al* found 90% of CKD patients with CAC and 25 % in control group[10].

Severity of calcification was significantly associated with decline in renal function. In the study of Hyun *et al* (KNOW-CKD), low eGFR was independently associated with CAC (p = < 0.001)[11]. Roy *et al* also found that patients with mild CKD had 2.2 times and moderate CKD had 6.4 times respectively, more CAC than the group with normal eGFR[12]. CAC is also associated with age, but we didn't find such association in our study. The probable reason for this may have been a small sample size. In present study, both CRP and homocysteine were significantly associated with CAC score. These markers have both atherogenic and thrombogenic effects that cause endothelial dysfunction by increasing oxidative stress, decreasing nitric oxide release and impairing vasodilatation. They also facilitate the differentiation of VSMCs into osteoblastic cells[13-15]. Kochi *et al* observed that the adjusted hazard ratios (HR, 95% confidence interval) for CVD were 1.88 (0.25 - 9.44) for patients with CKD with low CRP and 9.71 (3.27 - 31.97) for those with CKD with high CRP[16]. Cohen *et al* also found that homocysteine concentration was increased with a decline in eGFR (p = < 0.0001) and was significantly associated with CVD[17].

Hypertension is also an independent risk factor for CVD and CAC and it contributes to CAC by vascular remodelling and arteriosclerosis. The RAAS system also contributes to the pathogenesis of calcification. Amouei *et al* conducted a study and found hypertension in 40% and 62% of patients with CAC score ≤ 100 and CAC score > 100, respectively (P value = < 0.01)[18]. Chen *et al* also found that patients with CAC had higher systolic and diastolic BP than normal coronary arteries (p value = < 0.001)[19]. Hypoalbuminaemia was also significantly associated with CAC. The serum albumin is an imperative for maintaining oncotic pressure and microvascular integrity, regulating metabolic and vascular functions, providing binding ligands for substances, antioxidant activities, and anticoagulant effects. It is also involved in the vasodilatory response to NO[20]. Verma *et al* also found that serum albumin was significantly associated with aortic calcification. In their research the mean value of serum albumin (g/dL) was 3.93 ± 0.51 with aortic calcification index (ACI) < 20% and 3.56 ± 0.59 with ACI > 20% (p value = 0.0001)[21]. Anaemia also contributes to CVD and CAC. It causes medial wall calcification and osteoblastic transformation of VSMCs. Iron also plays an essential role in the oxidative phosphorylation in myocytes. Its deficiency causes cardiac remodelling and myocyte damage[22]. Mizuiri *et al* conducted a study to establish an association between iron deficiency anaemia and CAC and found that serum iron and transferrin saturation (TSAT) levels were significantly lower in patients with CAC score ≥ 400 than in those with CAC score < 400 (odds ratio 0.46, p = < 0.05)[23].

Abnormal mineral-bone metabolism was associated with higher CAC. The mechanism involved in this; abnormal mineral metabolism induces transdifferentiation of VSMCs into osteoblastic phenotype by Osf2/Cbfa1. They also accelerate the expression of osteochondrogenic markers such as Runx2, osterix, osteopontin, and alkaline phosphatase. Many studies are consistent with this association[24-26.] Russo *et al* found that higher serum concentration of phosphorus was significantly associated with greater progression of CAC[27]. Han *et al* also concluded that serum calcium, serum phosphorus and iPTH were significantly associated with CAC (p value = < 0.0001)[28]. Verma *et al* also found significant association of serum phosphorous level with vascular calcification (p value = < 0.0001)[29]. Bore *et al* found that iPTH was inversely related to eGFR (P = < 0.0001); they also found that, after adjusting for age and diabetes, iPTH was associated with myocardial infarction (OR, 1.6; 95% CI, 1.1 to 2.3 per unit natural log PTH) and congestive heart failure (OR, 2.0; 95% CI, 1.3 to

2.9 per unit natural log PTH)[30].

Insulin resistance is common in CKD patients and HbA1C can be used as a reliable marker of insulin resistance and in present research it was significantly associated with a higher CAC score. It accelerates the process of atherosclerosis by inducing oxidative stress, low-grade inflammation and endothelial dysfunction. The main mechanism involved in CAC includes an increase expression of bone matrix proteins such as osteopontin, type I collagen and alkaline phosphatase in the medial layer of blood vessels. It also accelerates the face of the osteoblast transcription factors-like RUNX2, BMP-2 and osteocalcin. Cavero-Redondo *et al* conducted a study and found that HbA1C was a reliable risk marker for cardiovascular mortality in both diabetics and non-diabetics[31]. Chen *et al* also found that HbA1C was increased with increasing calcification (p value = < .001)[19].

In the present study, dyslipidemia was significantly associated with a high CAC score, a predictor of CVD. Non-HDL lipids are highly atherogenic and leading to thrombogenesis by fibrinolysis inhibition while HDL lipids hamper atherosclerosis by cholesterol transport from the arterial wall to the liver for further excretion, inhibition of inflammation, platelet adhesion and LDL oxidation, etc. The mechanism behind collaboration of lipids with CAC is that non-HDL lipids and oxidized lipids enhance pro-calcific activity and mineralisation of vascular cells. It has a direct effect on both bone-forming and bone-resorbing cells. Alamgir *et al* found that the normolipidemic group had the highest percentage of individuals with normal vessels (60%) than hyperlipidemia group who had the highest rate of three vessels and four vessels calcification[32]. Elraoof *et al* found that dyslipidemia was associated with high CAC score[10]. Chen *et al* found that dyslipidemia was more strongly associated with CAC score > 100 than CAC score < 100 (p value = < 0.001)[33].

In the present research, significant correlation of CAC was found with eGFR, hypoalbuminaemia, abnormal mineral metabolism (hypocalcaemia, hyperphosphataemia, iPTH and product of calcium and phosphorous), CRP, homocysteine, HbA1C, dyslipidemia (decreased HDL, increased LDL, increased total cholesterol and increased triglyceride). This association was also significant on univariate regression analysis. But on performing multivariate regression analysis, only triglyceride, serum homocysteine, CRP, i-PTH, eGFR were significant after adjusting for confounding factors.

There were some limitations of this study. First, the cross-sectional study design does not establish a causal relationship between CAC and CKD and prospective studies are required for the same. The sample size of the study was only 130, including the control group. A larger sample size would have allowed a more accurate justification of the results of our research. Our study population had 30% of diabetic patients. As diabetic patients frequently have a high prevalence of cardiovascular disease, this could have been a confounding factor in our study. Due to the high prevalence of anaemia in CKD patients, HbA1C could be falsely low. Patients taking uric acid lowering agents, phosphate binders or erythropoietin stimulating agents, were not excluded.

As per study results, CAC has marked prevalence in CKD patients and being a surrogate marker of CVD, it indicates the increase risk of cardiovascular events in CKD patients. It is affected not only by abnormal mineral metabolism but also by many traditional and non-traditional risk factors such as hypertension, insulin resistance, dyslipidemia, anaemia, hyperhomocysteinaemia, CRP, uric acid, hypoalbuminaemia etc. Although CAC is more pronounced in dialysis patients, it also has prognostic significance in predialysis patients. So, by assessing CAC score high-risk patients can be distinguished early in course of disease, facilitating timely intervention and thereby preventing complications and improving quality of life in CKD patients. Follow-up and interventional studies are required for further evaluation and establishing a strong association between CAC as a major prognostic/predicting factor for CVD in CKD patients.

Conclusion

In conclusion, cardiovascular morbidity and mortality in CKD patients has become a huge problem in our country. Present study's results suggest that risk factors of CVD are highly prevalent in CKD patients. Cardiac calcification should be considered a marker of CVD risk in CKD patients and it improves risk prediction for CVD. It is also independently and significantly related to the dangers of cardiovascular disease. Various traditional and non-traditional risk factors such as increased CRP, iPTH, homocysteine, anaemia, hyperphosphataemia, dyslipidemia, insulin resistance, etc., accelerate the rate of cardiac calcification.

Further studies are warranted to determine measures to retard the rate of progression of cardiac calcification. The severity of CAC, dyslipidemia and HbA1C is negatively associated with kidney function. Present study showed that CAC score, lipid profile and HbA1C are reliable markers for screening CVD in CKD patients. Early diagnosis of CVD can help in aggressive management and hence decrease the morbidity and mortality associated with CKD. Several novel therapies to reduce the risk of CVD in CKD are in clinical development or have been already established, raising the hope that cardiovascular risk in patients with CKD may be modifiable in the future. So, the patients with even mild CKD who are not yet on haemodialysis, should be screened and treated more aggressively for heart disease than the average population.

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ORIGINAL ARTICLE

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A Comparative Study of Pulmonary Function Tests Between Obese and Non-Obese Asthmatic Patients

Akanksha Singh, Piyush Jain**, AK Malhotra****

Abstract

***Background:** Bronchial asthma and obesity are both chronic diseases constituting important health problems worldwide. Obesity and bronchial asthma are common conditions characterised by the presence of inflammation. This systemic pro-inflammatory state leads to worsening of the airway inflammation seen in asthmatic patients.*

***Materials and methods:** This is a cross-sectional observational study with the aim of assessing the effect of obesity on pulmonary function in asthmatic patients. Study was conducted in the Department of Medicine, PGIMER, Dr. Ram Manohar Lohia Hospital, New Delhi from November 2014 to March 2016. Asthmatic patients attending the Medicine Department who satisfied the inclusion and exclusion criteria were included in the study. We compared pulmonary function tests between obese and non-obese asthmatic patients and compared the severity of asthma between obese and non-obese asthmatic patients. Spirometry of the patients was done to evaluate the pulmonary function. They were assessed for asthma severity according to GINA (Global initiative for asthma) 2014 guidelines. Significance of difference in means (quantitative variables) was calculated using student t test. Significance of difference in proportions (qualitative variables) was calculated using chi square test.*

***Results:** The mean age of non-obese asthmatic patients was 38.76 years (SD±13.44). The mean age of obese asthmatic patients was 39.23 years (SD ± 9.03). The mean FEV1 (% predicted) of non obese asthmatic patients was higher than the mean FEV1 (% predicted) of obese asthmatic patients and it was statistically significant (p value 0.002). The mean FEV1/FVC ratio of non-obese asthmatic patients was higher than the mean FEV1/FVC ratio of obese asthmatic patients and it was statistically significant (p value < 0.001). There was statistically significant (p value < 0.001, df-3) difference in severity of asthma in non obese and obese asthmatic patients.*

***Conclusion:** There is a strong association between BMI and pulmonary function in asthmatic patients. Pulmonary function tests are more deranged in obese asthmatics patients in comparison to non-obese asthmatic patients. Also the pulmonary functions had a negative correlation with BMI, i.e., the pulmonary functions deteriorated with increasing BMI among asthmatic patients. In conclusion, overweight and obesity is associated with significantly more airflow obstruction and poor disease control.*

***Key words:** Asthma, obesity, pulmonary function test.*

Introduction

Bronchial asthma and obesity are both chronic diseases constituting important health problems worldwide. Bronchial asthma is a chronic inflammatory disease characterised by lower airway hyper responsiveness and by variable airflow limitation that can resolve spontaneously or through treatment[1].

The mechanisms linking obesity and asthma remain poorly understood and multiple hypotheses have been proposed. Some studies propose that bronchial asthma in obese subjects may have a particular phenotype[2]. Obesity may be associated with respiratory symptoms via cardio-respiratory de-conditioning, physiological restriction of the chest wall by excess mass, or comorbidities, including gastro-oesophageal reflux and sleep-disordered breathing[3].

Obesity and bronchial asthma are common conditions characterised by the presence of inflammation[4]. Numerous cytokines, and soluble fractions of their receptors and chemokines have all been found to be increased in obesity[5]. This systemic pro-inflammatory state leads to worsening of the airway inflammation seen in asthmatic patients[6].

Therefore, this study aims to find the probable association between obesity and asthma. It also aims to research the

**Assistant Professor, **Professor, Department of Medicine, ABVIMS, Atal Bihari Vajpayee Institute of Medical Sciences , Baba Kharak Singh Marg, New Delhi - 110 001; ***Former Professor and Consultant, Department of Medicine, Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 110 001.*

***Corresponding Author:** Dr Akanksha Singh, Assistant Professor, Department of Medicine, ABVIMS, Atal Bihari Vajpayee Institute of Medical Sciences and Dr RML Hospital, Baba Kharak Singh Marg, New Delhi - 110 001. Tel: 9811859379, E-mail: drakanksha777@gmail.com.*

impact of obesity on the clinical manifestations and severity of bronchial asthma which will be classified according to the GINA (Global Initiative for Asthma) guidelines[7].

Materials and methods

The aim of the study was to assess the effect of obesity on pulmonary function in asthmatic patients.The study was conducted with the objectives to compare pulmonary function tests and the severity of asthma between obese and non-obese asthmatic patients.

It was a cross-sectional observational study conducted from November 2014 to March 2016. Asthmatic patients attending the Medicine Department who satisfied the inclusion and exclusion criteria were included in the study. The diagnosis of asthma was based on history of characteristic symptom patterns (wheeze, dyspnoea, coughing) which are variable, both spontaneously and with treatment. Evidence of variable airflow limitation was demonstrated from bronchodilator reversibility testing (In adults: increase in FEV1 > 12% and > 200 mL). A patient with an FEV1 < 80% was given inhaled short acting beta-2 agonist bronchodilator and the spirometry was repeated after 15 min. If the FEV1 increases by more than 12%, it is indicative of reversible airway disease[8].

Inclusion criteria were subjects aged 18 years or older and who were asthmatic for more than one year. Exclusion criteria were subjects who were pregnant, had any additional respiratory disease (like COPD, ILD), smokers, with cardiac illnesses and patients who will not be able to perform pulmonary function tests by spirometry. Any cardiac illness was excluded by ECG and 2D-ECHO among all the patients included in the study.

A total of 30 obese asthmatics and 30 non-obese asthmatic patients were enrolled for the study. BMI of these patients was calculated and they were classified as obese and non-obese accordingly.

Spirometry of the patients was done to evaluate the pulmonary function. They were assessed for asthma severity according to GINA[7] (Global initiative for asthma) 2014 guidelines (Table I).

A patient was classified as underweight, overweight, and obese according to Body Mass Index (BMI) which is a simple index of weight-for-height used in adults (Table II). It is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2)[9].

Table I: GINA classification of bronchial asthma severity[7].

	Symptoms/Day	Symptoms/Night	PEF or FEV_1
Intermittent	< 1 time a week Asymptomatic and normal PEF between attacks	</= 2 times a month	>/= 80%
Mild Persistent	> 1 time a week but < 1 time a day Attacks may affect activity	> 2 times a month	>/=80%
Moderate Persistent	Daily Attacks affect activity	> 1 time a week	60% - 80%
Severe Persistent	Continuous Limited physical activity	Frequent	</= 60%

PEF: Peak Expiratory Flow; FEV_1: Forced Expiratory Volume in the first second.

Table II: Classification of patients according to BMI[9].

Classification	BMI (kg/m^2)
	Principal cut-off points
Underweight	< 18.50
Normal range	18.50 - 24.99
Overweight	≥ 25.00 - 29.99
Obese	≥ 30.00

Statistical analysis

The data were checked for normal distribution. The qualitative variables were described in the form of proportions and quantitative variables were described in the terms of mean scores, range and standard deviation. Significance of difference in means (quantitative variables) was calculated using student t test. Significance of difference in proportions (qualitative variables) was calculated using chi square test. Fischer exact test was applied in some of the statistical analysis. Correlation was also evaluated in some cases. A p value of less than 0.05 was considered significant.

Results

The study included 30 non-obese and 30 obese asthmatic patients. Among non obese asthmatic; 7 (23.33%) patients were between the age of 18 - 30 years; 9 (30%) were in age group of 31 - 40 years; 7 (23.33%) were in the age group of 41 - 50 years; 5 (16.6%) were between 51 - 60 years of age and only 2 (6.66%) were above the age of 60 years. Among non-obese asthmatic patients, 14 (46.66%) were women and 16 (53.33%) were men.

Among obese asthmatic patients, 5 (16.66%) were in the

age group of 18 - 30 years; 13 (43.33%) were of age between 31 - 40 years; 8 (26.66%) were in age group of 41 - 50 years; and 4 (13.33%) between the age of 51 - 60 years. No obese asthmatic was above the age of 60 years. Among obese asthmatic patients, 11 (36.66%) were women and 19 (63.33%) were men.

The number of patients in various age groups of non-obese and obese asthmatic patients was comparable with a p value of 0.51 (df = 4), which was not statistically significant. Fig. 1 show the distribution of non-obese and obese asthmatic patients according to age.

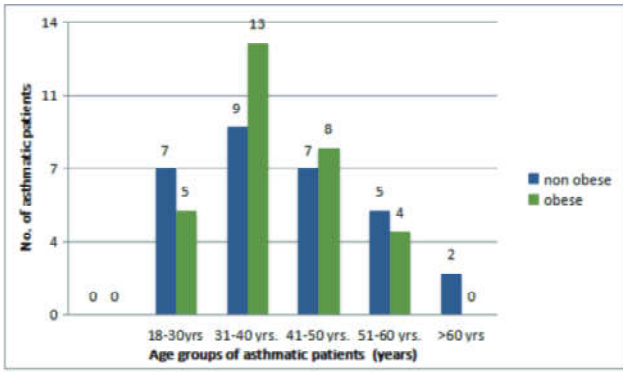

Fig. 1: *Distribution of non-obese and obese asthmatic patients according to age.*

The mean age of non-obese asthmatic patients was 38.76 years (SD ± 13.44). The mean age of obese asthmatic patients was 39.23 years (SD ± 9.03). The difference in the mean age of non-obese and obese asthmatic patients was not statistically significant (p value 0.87).

The mean BMI of non-obese asthmatic patients was 23.47 kg/m^2 (SD ± 3.35). The mean BMI of obese asthmatic patients was 30.96 (SD ± 0.51). There was a statistically significant difference in mean BMI of non-obese and obese asthmatic patients (p value < 0.001). Fig. 2 show the distribution of asthmatic patients according to Body Mass Index (BMI) (kg/m^2).

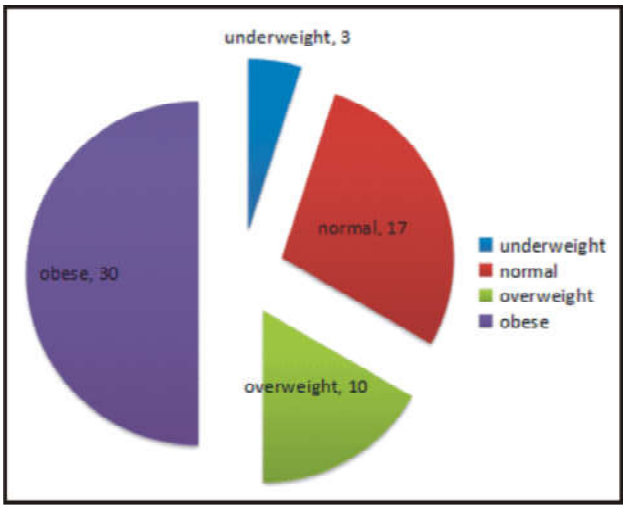

Fig. 2: *Distribution of asthmatic patients according to BMI.*

The mean FEV1 (% predicted) of non-obese asthmatic patients was 65.56 (SD ± 5.37). The mean FEV1 (% predicted) of obese asthmatic patients was 61.56 (SD ± 3.85). The mean FEV1 (% predicted) of non-obese asthmatic patients was higher than the mean FEV1 (% predicted) of obese asthmatic patients and it was statistically significant. (p value 0.002). Fig. 3 shows the mean FEV1 (% predicted) of non-obese and obese asthmatic patients.

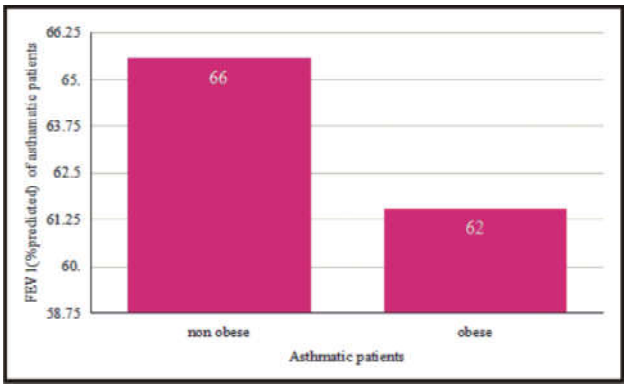

Fig. 3: *Mean FEV1 (% predicted) of non-obese and obese asthmatic patients.*

The mean FVC (% predicted) of non-obese asthmatic patients was 70.66 (SD ± 5.95). The mean FVC (% predicted) of obese asthmatic patients was 65.70 (SD -4.40). Thus, mean FVC (% predicted) of non-obese asthmatic patients was higher than that of obese asthmatic patients and it was statistically significant (p value < 0.001). Fig. 4 shows the distribution of asthmatic patients according FVC (% predicted).

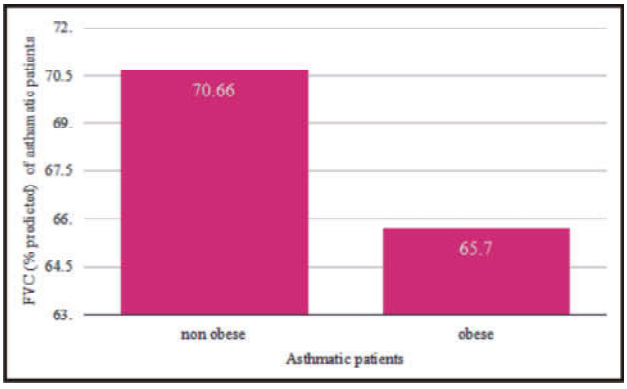

Fig. 4: *Mean FVC (% predicted) of non-obese and obese asthmatic patients.*

The mean FEV1/ FVC ratio of non-obese asthmatic patients was 66.26 (SD ± 4.55). The mean FEV1/FVC ratio of obese patients was 61.66 (SD ± 4.38). The mean FEV1/FVC ratio of non-obese asthmatic patients was higher than the mean FEV1/FVC ratio of obese asthmatic patients and it was statistically significant (p value < 0.001). Fig. 5 displays mean FEV1/FVC ratio of non-obese and obese asthmatic patients.

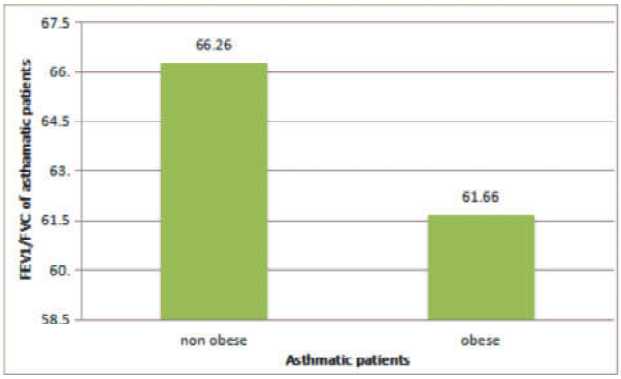

Fig. 5: *Mean FEV1/FVC ratio of non-obese and obese asthmatic patients.*

The Fig. 6 depicts the correlation of BMI (kg/m^2) with FEV1 (% predicted) of asthmatic patients. There was statistically significant correlation between BMI (kg/m^2) and FEV1 (% predicted) of asthmatic patients [Pearson correlation co-efficient = (-0.45); p value < 0.001; df-1]. As the BMI of asthmatic patients increased, the FEV1 (% predicted) decreased, i.e., a negative correlation was observed.

There was statistically significant correlation between BMI (kg/m^2) and FVC (% predicted) of asthmatic patients [Pearson correlation co-efficient (-0.41); p value 0.001; df-1]. As the BMI of asthmatic patients increased, the FVC (% predicted) decreased, i.e., a negative correlation was observed. The Fig. 7 depicts the correlation of BMI (kg/m^2) with FVC (% predicted) of asthmatic patients.

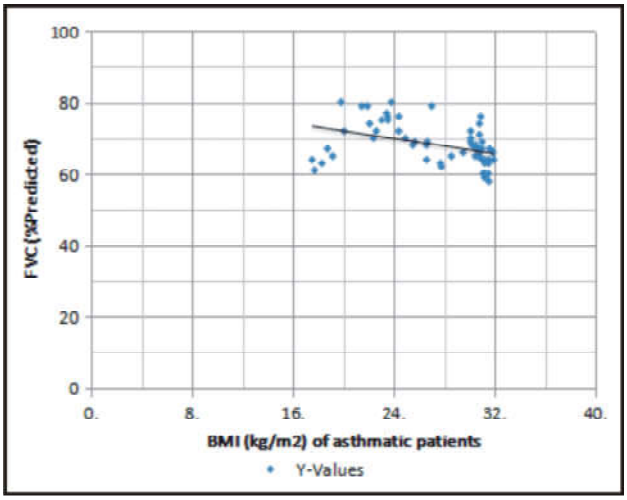

Fig. 7: *Correlation of BMI (kg/m^2) of asthmatic patients with FVC (% predicted).*

There was statistically significant correlation between BMI (kg/m^2) and FEV1/FVC (% predicted) of asthmatic patients [Pearson correlation co-efficient (-0.55; p value < 0.001, df-1]. As the BMI of asthmatic patients increased, the FEV1/ FVC decreased, i.e., a negative correlation was observed. The Fig. 8 depicts the correlation of BMI (kg/m^2) with FEV1/ FVC of asthmatic patients.

Fig. 9 depicts the severity of asthma in non-obese and obese asthmatic patients. There was statistically significant (p value

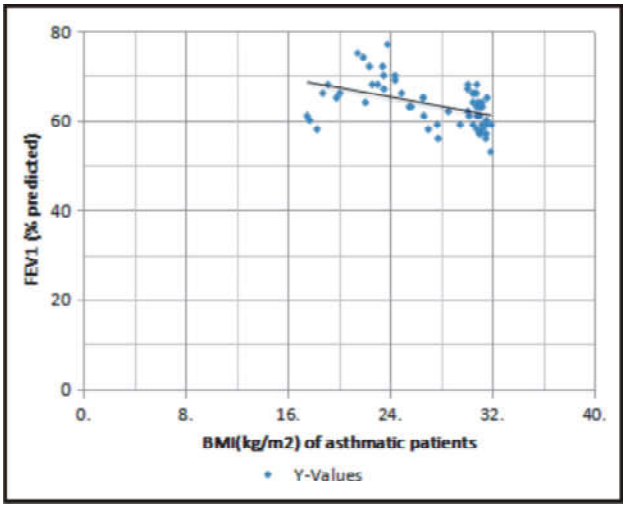

Fig. 6: *Correlation of body mass index with FEV1 (% predicted) in asthmatic patients.*

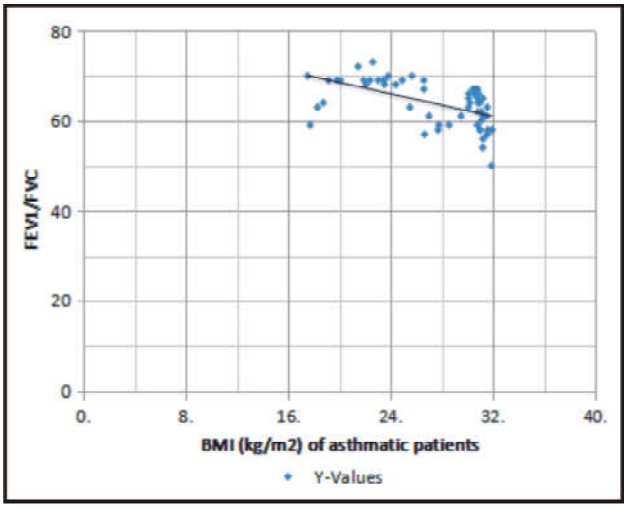

Fig. 8: *Correlation of BMI (kg/m^2) of asthmatic patients with FEV1/FVC ratio.*

< 0.001, df-3) difference in severity of asthma in non-obese and obese asthmatic patients. Among non-obese asthmatic patients, 23 (76.66%) patients had grade 3 severity of asthma, whereas 7 (23.33%) had grade 4 severity of asthma. Among obese asthmatic patients, 16 (5.33%) had grade 3 severity of asthma, while 14 (46.66%) had grade 4 asthma. Proportion of patients with grade 4 severity of asthma was significantly higher in obese asthmatic patients in comparison to non-obese asthmatic patients.

Table III: Severity of asthma in obese and non-obese asthmatic patients

S. No.	Asthmatic patients	Severity of asthma		p value (df)
		Grade* 3	Grade* 4	
1.	Non obese (n=30)	23 (76.66%)	7 (23.33%)	< 0.001 (3)
2.	Obese (n=30)	16 (53.33%)	14 (46.66%)	

**Grade: GINA classification, *chi square test*

Fig. 9 shows the association of BMI (kg/m^2) with Peak expiratory flow rate (% predicted) in asthmatic patients. There was statistically significant difference in the PEF (% predicted) of obese and non-obese asthmatic patients. It was significantly higher in non-obese (68.26 ± 3.26) asthmatic patients in comparison to obese (68.26 ± 3.76) asthmatic patients (p value < 0.001).

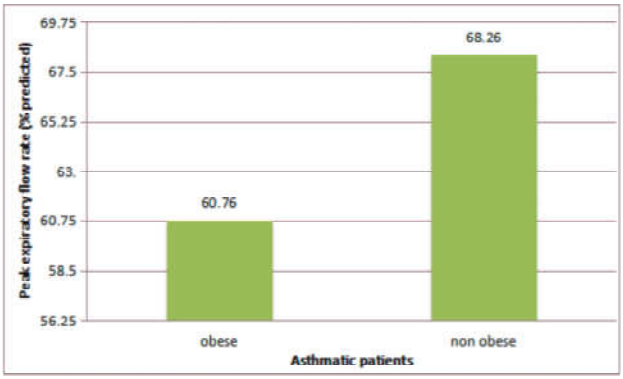

***Fig. 9:** Association of BMI (kg/m^2) with Peak expiratory flow rate (% predicted) in asthmatic patients.*

Discussion

Bronchial asthma and obesity are both chronic inflammatory diseases and each one is associated with systemic inflammatory state. Both of them affect respiratory mechanics and pulmonary function to variable extent.

Taking this into consideration, the present study was conducted in 30 obese asthmatic patients and 30 non-obese asthmatic patients. Pulmonary functions were assessed and compared in the two groups.

The mean age of non-obese asthmatic patients was 38.76 years (SD ± 13.44). The mean age of obese asthmatic patients was 39.23 years (SD ± 9.03). Thus the mean age of both age groups was comparable.

In the current study, the mean FEV1 (% predicted) of non-obese asthmatic patients was higher than the mean FEV1 (% predicted) of obese asthmatic patients. This was found to be statistically significant in current study. Similar results were found in a cross-sectional study conducted between 2009 and 2010 in a Respiratory Hospital in Tunisia by Maalej *et al*[10] (2012) in which it was observed that mean FEV1 was significantly lower in the obese group.

A statistically significant difference in the mean FVC (% predicted) of non-obese and obese asthmatic patients was observed in the current study. The mean FVC (% predicted) was significantly lower in obese asthmatic patients in comparison to non-obese asthmatic patients. Spathopoulos *et al*[11] (2009) conducted a study aimed to investigate primarily the effect of obesity on the lung function tests and secondary the possible link of obesity with atopy and asthma in a large cohort of children in Greece. The % expected FVC was significantly reduced in overweight or obese children compared to children with normal weight (p value < 0.001) as observed in our study.

The current study also revealed statistically significant difference in the mean FEV1/FVC ratio (% predicted) of non-obese and obese asthmatic patients. It was found to be higher in non-obese asthmatic patients in comparison to obese asthmatic patients. Similarly, in a study conducted by Lang[12] (2013) in obese and non-obese males, it was observed that obese males had significantly reduced FEV1/FVC. Similar results were obtained in a study carried-out by Chu *et al*[13] (2009) to find the relationship between body mass index (BMI) and lung function. This study showed that high BMI in both sexes was associated with low FEV1/FVC.

It was also observed in the current study, that not only there was statistically significant difference in mean FEV1 (% predicted) and mean FVC (% predicted) between non-obese and obese patients, but also a significant decrease in FEV1 (% predicted) and FVC (% predicted) as the BMI of patients increased. There was statistically significant correlation between FEV1 (% predicted) and FVC (% predicted) with BMI of asthmatic patients. Similar results were observed in a study conducted by Raviv S, Dixon EA, Kalhan R, Shade D and Smith JL[14] (2011) in America to

determine effect of obesity on asthma severity. The study revealed decreasing FEV1 and FVC with increasing BMI.

Also, a statistically significant negative correlation was observed between BMI (kg/m^2) and FEV1/FVC (% predicted) among asthmatic patients.

A statistically significant (p value < 0.001, df-3) difference in severity of asthma was observed between non-obese and obese asthmatic patients in the present study. The proportion of patients with grade 4 asthma severity was more in obese asthmatic group in comparison to non-obese asthmatic group. A study was conducted by Fitzpatrick S, Joks R and Silverberg JI[14] among inner city adults at a bronchial asthma clinic in New York between 1997 and 2010 to determine whether or not obesity is associated with increased bronchial asthma prevalence, severity and exacerbations. The study revealed that class I and II/III obesity was associated with worsened asthma severity (ordinal logistic regression; I: OR: 4.23, 95% CI: 1.61 - 11.06, P = 0.003; II/III: OR: 2.76, 95% CI: 1.08 - 7.09, P = 0.03), as observed in the current study.

In the current study, there was statistically significant difference in the PEF (% predicted) of obese and non-obese asthmatic patients. It was significantly higher in non-obese (68.26 ± 3.26) asthmatic patients in comparison to obese (68.26 ± 3.76) asthmatic patients (p value < 0.001). Borse JL, Modak KH, Bansode GD, Yadav DR[16] (2014) carried-out a study among first year medical college student to find the effect of body weight on PEFR (Peak Expiratory Flow Rate). Findings of the study suggested that PEFR values of overweight student were significantly less compared to normal weight, as observed in the current study.

This study shows that there is a strong association between BMI and pulmonary function in asthmatic patients. Pulmonary function tests are more deranged in obese asthmatics patients in comparison to non-obese asthmatic patients. Also, the pulmonary functions had a negative correlation with BMI, i.e., the pulmonary functions deteriorated with increasing BMI among asthmatic patients. Thus, in conclusion, overweight and obesity is associated with significantly more airflow obstruction and poor disease control.

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ORIGINAL ARTICLE

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Effect of Attitude and Transportation on Healthcare Utilisation Among Geriatric Patients of Respiratory Diseases – An Indian Perpective - Delhi NCR and Ghaziabad

Sonisha Gupta, Smita Asthana**, Atul Kumar Gupta***, Pawan Kumar Basarwadia*****

Abstract

Background: Ageing is associated with gradual decline in all body functions and compromised host defence mechanisms. Hence, with increasing age, the elderly develop a number of health problems.

Methods: It was a cross-sectional study, conducted in urban and rural areas of Delhi NCR (National Capital Region) and Ghaziabad district of Uttar Pradesh. People aged 60 yrs and above residing in the sample area were included. Elderly with respiratory diseases were asked to fill a questionnaire regarding health care utilisation.

Results: Attitude of elderly towards health issues in old age has significant effect on their healthcare utilisation. Presence of 'fear of addiction' in elderly decreased healthcare utilisation from 42.1% to 4.1% in urban and from 16.6% to 5.2% in rural elderly. Similarly, only 5.1% urban and 0.9% rural elderly who thought that disease was 'part of old age and medicines will not help' were utilising healthcare services. Presence of attitude that 'Problem not serious, medicines not needed' decreased healthcare utilisation from 39.6% to 3.5% in urban and 15.1% to 1.3% in rural elderly.

In our study 'availability of vehicle' had significant effect on healthcare utilisation by urban as well as rural elderly (p value = .031 and .001 respectively). 'Inconvenient transportation' did not affect healthcare utilization significantly by either urban or rural elderly but it significantly affected healthcare utilisation (p value = .019) in combined data. 'Need of more than one companion' for going to healthcare centre was associated with lesser healthcare utilisation in both urban (18.2% vs 33.9%) as well as rural elderly (0% vs 11.6%).

Conclusion: Majority of the elderly have a negative attitude regarding utilisation of healthcare services. They find transportation to a healthcare facility as inconvenient and time consuming. This negative attitude as well as transportation problems are significantly more with the rural elderly than their urban counterparts.

Key words: Elderly, attitude, transportation, healthcare utilisation, respiratory diseases, geriatric.

Introduction

Various theories proposed to explain biological basis of ageing are: immune theory, neuroendocrine theory, free radical theory, cell ageing theory, somatic mutation theory, error theory[1]. Immune system is also affected by ageing. Ageing related functional changes in the organs make them vulnerable to infections. Almost all organ systems of body are affected.

All over the world, the number of elderly is progressively increasing both in developed and developing countries. Currently, the growth rate of the older population (1.9 %) is significantly higher than that of the total population (1.2 %)[2].

In India also, the number of elderly is progressively increasing. It is expected to increase up to 12% by 2026 (173 million) and approximately 20% (316 million) by 2050[3]. In India also there is a wide variation in the proportion of elderly in different states. While in Kerala they constituted 11% of total population, in Uttar Pradesh they were 6% only. By 2026 it is expected to increase to 18% and 10% respectively.

This demographic change is a result of the combined impact of increasing life expectancy and declining fertility. Life expectancy at birth in India increased from 37 years in 1950 to 65 years in 2011[4]. During the same period, fertility rates in India have declined from 5.9 to 2.6 children per women[5].

A steep rise in the world population of elderly in the last five decades has compelled policy makers, health planners,

Professor, Department of Respiratory Medicine, SMSR Sharda University, Greater Noida - 201 308, Uttar Pradesh. **Scientst E, ICMRNICPR, Greater Noida - 201 308, Uttar Pradesh, ***Associate Professor, Department of Surgery, Government Institute of Medical Sciences, Greater Noida - 201 308, Uttar Pradesh,**Chest Physician, Department of Pulmonary Medicine, ABVIMS and Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 110 001.*

Corresponding Author: Dr Sonisha Gupta, Professor, Department of Respiratory Medicine, SMSR Sharda University, Greater Noida - 201 308, Uttar Pradesh. Tel: 8527214749, E-mail: sonishagupta@gmail.com.

economists and demographers to pay greater attention to Gerontology.

Healthcare infrastructure is as such inadequate in India. But even that is not utilised properly by elderly. Elderly are a unique population subgroup with their own problems in utilizing available healthcare services. A number of Indian as well as international investigators have tried to understand the factors affecting utilisation of healthcare services by the elderly. Despite respiratory diseases being a significant cause of morbidity in the elderly, in our search we were able to find only one Indian study by Sudha *et al* which focused on healthcare utilisation by elderly for respiratory disease[6].

Issues related to awareness, attitude towards old age problems, and the need to address them is another obstacle in tackling the old age problems in our country. Hence, it is important to understand the special needs of elderly, obstacles to the utilisation of available healthcare facilities in the form of attitudinal barrier, difficulties encountered in utilisation, shortcomings of the present systems from the perspective of end user. The fact that disease and disability are not a part of old age should be emphasized and health problems should be addressed.

Methodology

A descriptive survey of geriatric population aged 60 yrs and above was conducted in urban and rural areas of Delhi NCR (National Capital Region) and Ghaziabad district of Uttar Pradesh. Urban colonies and rural villages, conglomerated in closed areas were selected on the basis of convenience of sampling from each urban and rural selected units, by systematic random sampling. Elderly in every alternate household was interviewed and adequate sample size was achieved.

Population Setting: Urban area – Nandgram is a locality in Ghaziabad city. There are more than 10,000 houses with 7 blocks and free households. It is inhabited mainly by lower middle class families. Rural area – six selected villages were – Chipiyana Buzurg and Shah beri from Greater Noida, Chhaprauala and Shahpur Bamheta from one side while Ilaichipur and Khanpur from the other end of Ghaziabad.

Sample of 51 elderly from Shah Beri village, 343 from Chipyana, 405 from Chhapraula, 136 from Shahpur Bamheta, 495 Ilaichipur and 73 was collected Khanpur village. A sample of 1,503 from rural and 1,522 from urban areas was collected. Total combined sample was 3,025.

Period of study: January 2015 to January 2018.

Sample size: For qualitative data, the formula used to derive sample size is: $n = 4\ pq/L^2$. (p - prevalence) available literature on prevalence of respiratory illness among elderly was assumed as 20% with an allowable error of 3% in a range of 17% - 23%. By simple random sampling for a given prevalence with 95% confidence level, a sample size of 682 was required. Attrition of 10% was added to 682 and then sample size was fixed at 750. As our sample procedure was systematic, we double the size and fix it at 1,500 each in rural and urban groups. It was predicted to give an average of 300 respiratory cases of elderly in each group.

Tools and Methodology

Door-to-door survey was conducted. A pre-designed, pre-tested questionnaire having 2 parts was used. First part included socio-demographic characteristics, self-reported co-morbidities and physical disabilities. Three healthcare workers were trained. Previous all medical records of patients were seen by them. After analyzing screening proforma, elderly with suspected respiratory disease were selected. In the second stage, screening proformas of suspected cases were verified. General and respiratory system examination was carried-out. These patients with respiratory diseases were asked about attitude towards health services and transportation on healthcare utilisation.

Statistical analysis

Microsoft Excel 2010 was used for data entry. Statistical analysis was done using IBM SPSS v 20.0.0. and 23.0.0 both. Categorical variables were analysed using proportions and percentages. Firstly, a descriptive analysis was performed for all records (n = 3,025), both urban and rural separately. And then association between categorical variables was studied by two-way cross-tabulations and the significance established by Chi-square test. The level of statistical significance was assessed at (P values less than 0.05) 5% probability.

Effect of attitude and transportation on healthcare utilisation was analysed in both urban and rural groups separately. It was assessed by chi-square test. Association between these two groups among all variables was also established by chi-square test.

Odd ratio at 95% confidence intervals were used for strength of association and interpretation of bivariate analysis. If differences found significant on univariate analysis, then necessary further analysis of the data was conducted by controlling for demographic and health characteristics. Multiple regression analysis was used to analyse various factors for assessing their independent contribution after adjusting for various factors in the model.

Table I: Attitude vs healthcare utilisation.

Attitude		Healthcare utilisation														
		Urban					Rural					Combined				
		Inadequate	No	Yes	Total 100.0%	P value	Inadequate	No	Yes	Total 100.0%	P value	Inadequate	No	Yes	Total 100.0%	P value
Fear of addiction	Yes	6690.4%	45.5%	34.1%	73100.0%	<.001	10477.0%	2417.8%	75.2%	135100.0%	.007	17081.7%	2813.5%	104.8%	208100.0%	<.001
	No	10851.7%	136.2%	8842.1%	209100.0%		10665.0%	3018.4%	2716.6%	163100.0%		21457.5%	4311.6%	11530.9%	372100.0%	
Part of old age medicines will not help	Yes	3282.1%	512.8%	25.1%	39100.0%	<.001	8675.4%	2723.7%	10.9%	114100.0%	<.001	11877.1%	3220.9%	32.0%	153100.0%	<.001
	No	14258.4%	124.9%	8936.6%	243100.0%		12467.4%	2714.7%	3317.9%	184100.0%		26662.3%	399.1%	12228.6%	427100.0%	
Medicines not needed	Yes	5087.7%	58.8%	23.5%	57100.0%	<.001	5973.8%	2025.0%	11.3%	80100.0%	.002	10979.6%	2518.2%	32.2%	137100.0%	<.001
	No	12455.1%	125.3%	8939.6%	225100.0%		15169.3%	3415.6%	3315.1%	218100.0%		27562.1%	4610.4%	12227.5%	443100.0%	
Decided to handle problem by self	Yes	7783.7%	1010.9%	55.4%	92100.%	<.001	9982.5%	1512.5%	65.0%	120100.0%	<.001	17683.0%	2511.8%	115.2%	212100.0%	<.001
	No	9751.1%	73.7%	8645.3%	190100%		11162.%	3921.9%	2815.7%	178100.0%		20856.5%	4612.5%	11431.0%	368100.0%	
Available services not good	Yes	1368.4%	210.5%	421.1%	19100.%	.443	2472.7%	721.2%	26.1%	33100.0%	.562	3771.2%	917.3%	611.5%	52100.0%	.131
	No	16161.2%	155.7%	8733.1%	263100.0%		18670.2%	4717.7%	3212.1%	265100.0%		34765.7%	6211.7%	11922.5%	528100.0%	
Any Negative attitude	Absent	4536.0%	32.4%	7761.6%	125100.0%	<.001	2242.3%	611.5%	2446.2%	52100.0%	<.001	6737.9%	95.1%	10157.1%	177100.0%	<.001
	Present	12982.%	148.9%	148.9%	157100.0%		18876.4%	4819.5%	104.1%	246100.0%		31778.7%	6215.4%	246.0%	403100.0%	

Table 3: Transportation problems vs healthcare utilisation.

Transportation		Healthcare utilisation														
		Urban					Rural					Combined				
		Inadequate	No	Yes	Total 100.0%	P value	Inadequate	No	Yes	Total 100.0%	P value	Inadequate	No	Yes	Total 100.0%	P value
Inconvenient	Yes	8166.4%	64.9%	3528.7%	122100.0%	.363	16769.6%	4518.8%	2811.7%	240100.0%	.518	24868.5%	5114.1%	6317.4%	362100.0%	.019
	No	9057.3%	117.0%	5635.7%	157100.0%		4276.4%	814.5%	59.1%	55100.0%		13262.3%	199.0%	6128.8%	212100.0%	
Vehicle available	Yes	8854.3%	106.2%	6439.5%	162100.0%	.031	6758.8%	3228.1%	1513.2%	114100.0%	.001	15556.2%	4215.2%	7928.6%	276100.0%	<.001
	No	8370.9%	76.0%	2723.1%	117100.0%		14278.9%	2011.1%	1810.0%	180100.0%		22575.8%	279.1%	4515.2%	297100.0%	
Long time taken	Yes	11259.6%	126.4%	6434.0%	188100.0%	.627	17471.3%	4217.2%	2811.5%	244100.0%	.584	28666.2%	5412.5%	9221.3%	432100.0%	.985
	No	5964.8%	55.5%	2729.7%	91100.0%		3568.6%	1121.6%	59.8%	51100.0%		9466.2%	1611.3%	3222.5%	142100.0%	
Companion available	Yes	12461.7%	126.0%	6532.3%	201100.0%	.748	14771.4%	3918.9%	209.7%	206100.0%	.414	27166.6%	5112.5%	8520.9%	407100.0%	.956
	No	4760.3%	56.4%	2633.3%	78100.0%		6269.7%	1415.7%	1314.6%	89100.0%		10965.3%	1911.4%	3923.4%	167100.0%	
Need > 1 companion	Yes	1777.3%	14.5%	418.2%	22100.0%	.342	763.6%	436.4%	00.0%	11100.0%	.220	2472.7%	515.2%	412.1%	33100.0%	.717
	No	15459.9%	166.2%	8733.9%	257100.0%		20271.1%	4917.3%	3311.6%	284100.0%		35665.8%	6512.0%	12022.2%	541100.0%	

In the urban population, among factors related to attitude of elderly towards utilisation of healthcare services, 'fear of addiction to medicines' , 'medicines not needed' for their present illness, their illness being 'part of old age' , 'self handling' were found to be significant with p value of 0.001 (< 0.05). In transportation group, sex, socio-economic status, vehicle not available were found to be significant with p value of 0.036, 0.043, .010 respectively with ($p < 0.05$).

In the rural population, in attitude group, negative belief of 'fear of addiction', 'medicine not needed', 'part of old age' , 'self handling' were found to be significant with p value of 0.00 (< 0.05). In transportation group, no factor were found to be significant.

In the combined population, in attitude group, negative belief of 'fear of addiction', 'medicine not needed', 'part of old age', 'self handling' were found to be significant with p value of 0.00 and due to bad service of health sector ($p = .008$) (< 0.05). In transportation group socio-economic status, vehicle not available were found to be significant with p value of $p = .015$, $p = .006$ respectively with ($p < 0.05$).

Discussion

Attitudinal factors as barriers to healthcare utilisation by the elderly are unique to our society. Illiteracy, ignorance, poverty, socio-cultural conditioning lead to fatalistic attitude in old age. Old age has been described as a curse in ancient Indian literature. Many older people take ill health in their stride as a part of the usual/normal ageing. They feel not much can be done about it. They are resigned to bear with the ill effects of diseases. There are also many misconceptions about the need and effects of treatment. Besides, many of them also resort to non scientific methods.

In our study, only 44.3% (125/282) of urban and significantly lesser 17.4% (52/298, $p < .001$) rural elderly had no negative

Table III: Group wise regression analysis.

	Urban			
Attitude				
Factor	**Sig. (p value)**	**Odds ratio**	**(95% CI)**	
Sex	.084	1.600	.93 2.76	
Age	.558	1.261	.57	2.78
Educational status	.852	1.056	.59	1.90
S-E class	.183	1.441	.83	2.49
Fear of addiction	.001	5.158	2.38	11.17
Illness part of old age	.001	15.335	4.48	52.46
No need of medicines	.001	14.826	4.35	50.50
Decided to handle self	.001	3.991	1.90	8.40
Constant	.008	.260	.09	. 72
Transportation				
Sex	.036	1.999	1.03	3.87
Age	.112	1.809	.86	3.82
Educational status	.689	.873	.44	1.72
S-E class	.043	1.792	1.01	3.19
No vehicle available	.010	2.087	1.18	3.68
Constant	.155	.567	26	1.26
Rural				
Attitude				
Location	.106	1.561	.90	2.70
Sex1	.084	1.600	.93	2.76
Age	.558	1.261	.57	2.78
Educational status	.852	1.056	.59	1.90
S-E class	.183	1.441	.83	2.49
Fear of addiction	.000	5.158	2.38	11.17
Illness part of old age	.000	15.335	4.48 52.46	
No need of medicines	.000	14.826	4.35	50.50
Decided to handle self	.000	3.991	1.90	8.40
Constant	.008	.260	.09	.72
Combined (Urban and Rural data)				
Attitude				
Location	.177	1.463	.83	2.57
Sex	.071	1.650	.95	2.88
Age	.643	1.204	.54	2.68
Educational status	.830	1.066	.59	1.93
S-E class	.161	1.475	.85	2.57
Fear of addiction	.000	5.277	2.40	11.58
Illness part of old age	.000	16.182	4.70	55.70
No need of medicines	.000	16.088	4.70	55.04
Decided to handle self	.000	3.807	1.79	8.11
Avlbl. Services bad	.008	3.720	1.39	9.99
Constant	.008	.254	.09	.71
Transpotation				
Location	.000	3.270	2.06	5.20
Age	.352	1.346	.71	2.55
Educational status	.256	1.334	.80	2.22
S-E class	.015	1.745	1.10	2.76
Sex	.351	1.250	.77	2.02
No vehicle avlbl.	.006	1.838	1.18	2.87
Constant	.001	.242	.10	. 58

attitudinal factor regarding healthcare utilisation. It means that a large number of urban (55.7%) and rural elderly (82.6%) had at least one negative attitudinal factor. Although all negative attitudinal factors were more prevalent in rural population but only 'fear of addiction' and 'disease being part of old age thus requiring no treatment' achieved statistical significance ($p < .001$). This difference between urban and rural elderly is explainable due to difference in literacy, socio-economic status and socio-cultural milieu of two population.

Effect of negative attitude on healthcare utilisation has been well-documented in literature. In a study 39.6% elderly did not seek treatment for their illness due to their belief that it is part of old age, while 36.8% considered their morbidity as minor illness requiring no treatment[7]. Fear of discovering a serious illness and unneeded tests led 18.1% and 16.3% elderly respectively to avoid treatment in a rural Bengal study[8]. Sharma *et al* reported the most common reason for not seeking healthcare was the perception of disease as an age related phenomenon(49.6%)[9]. A study in Nepal found 'ignorance due to old age' and 'trust on God for healing' being reasons for not seeking treatment by 64% and 8% elderly respectively. Goswami *et al* also noted fatalistic attitude as a reason for not seeking any treatment by elderly[10].

In our study also, four attitudinal problems viz ' fear of addiction', 'part of old age medicines not needed', 'decided to handle problem self' and 'illness not serious so no need for medicines' had significantly negative effect on healthcare utilisation by both urban and rural elderly. Absence of any negative attitudinal factor had significant positive effect on utilisation ($p < .001$). In fact when regression analysis was done, absence of negative attitudinal factor was found to be the most significant factor affecting healthcare utilisation. These results are consistent with the literature as discussed above. This data emphasizes the need of creating awareness and bringing about attitudinal change in society as a whole, regarding problems of old age. Society also needs to be educated to treat old age as just another phase of life which can be enjoyed, provided all problems are properly addressed to.

Although perceived health status was a strong significant predictor of health service utilisation, the older persons who reported their health status as "fair" or "poor" were less likely to utilize health services than their counterparts[11]. This result agreed with a study in China reporting that older residents were less likely to utilize health services when they felt unwell[12]. Conversely, individuals with more negative self-perception of ageing were more likely to delay care and reported more reasons for delay[13]. Hence, attitudes toward one's ageing experience may influence health service utilisation.

A study by Maroof *et al* 2018,one of the reason for non-utilisation of health services was considering disease as normal part of ageing 13 (21.7 %) out of 60[14].

Accessibility of healthcare services is an important determinant of healthcare utilisation. In our study, distance of healthcare facilities in rural areas was significantly more than in urban areas ($p < .001$). While for 49% (139/282) urban residents healthcare facility was more than 3 km away for rural areas much higher proportion of elderly, 74% (221/298), had healthcare facility this much distant. This rural-urban difference is understandable since healthcare facilities are disproportionately concentrated in urban areas. Effect of geographical proximity of healthcare facility on healthcare utilisation has been studied extensively. In a rural Assam study, 27% respondents were not utilizing health care services due to facility being too far[15]. In a rural Bengal study as high as 48% elderly were not using healthcare services due to distance[16]. In a Shimla study also, 19% elderly cited long distance as the reason for non-utilisation[17]. Ahmed *et al* found geographical proximity to be a strong catalyst for healthcare seeking in Oman[18]. Similarly, a South African study found distance to health facilities a barrier for those wishing to access healthcare[19].

Not all studies have found effect of distance on healthcare utilisation. In Pakistan neither driving distance nor driving time showed association with the use of public health services for treatment of acute childhood illness[20]. Similarly no association was found between the use of contraceptive services and straight-line distances in Malawi[21]. No effect of distance on healthcare utilisation in these studies may be due to the fact that these studies concerned healthcare utilisation by young people for their own needs or of their children. Distance is likely to pose more difficulties for the elderly in utilisation of healthcare services in view of their own poor physical health and need of an accompanying person especially if distance to healthcare facility is more.

In the present study also, no significant effect of distance on healthcare utilisation was found. We had divided healthcare service distance in 3 categories, i.e., < 1 km, 1 - 3 km, > 3 km. Since availability of healthcare services is better in Delhi NCR, this small difference in distance is unlikely to make a significant effect on healthcare utilisation. Another reason could be that distance is only one part of transportation problem and availability of good transport facilities will negate the effect of this small difference in distance. Another Delhi-NCR study in Ballabhgarh, Faridabad found very few elderly reporting distance as a barrier to healthcare utilisation[10].

We studied transportation problems under five variables, i.e., inconveniece, long time taken, vehicle availability, availability of companion and need for more than one companion to acompany. Only a small number of elderly (5.7 - 33/580) reported the need of more than one companion to take them to a healthcare facility. In all other four, transportation problems were reported by a significant number of elderly especially in rural areas, which is understandable. Among the rural respondents, 80.5% (240/298) found transport to healthcare services inconvenient, 81.9% (244/298) complained they consume too much time. 60.4% (180/298) rural respondents said vehicle was not available while 29.9% (89/298) complained of non-availability of companion to take them to healthcare centre. When effect of all these factors was individually analysed on healthcare utilisation, availability of vehicle was found to have statistically significant effect on both rural ($p = .001$) and urban elderly ($p = .031$). Effect of inconvenience was found to be significant only when urban and rural data was combined. Other factors had no significant effect on healthcare utilisation. Probably availability of personal/public vehicle and to some extent convenience, compensates for other transportation problems.

Transportation problems are more likely to affect healthcare utilisation by the elderly in view of their weak body, illness, physical disabilities, and increasing need of a companion. This effect has been found by many investigators. Rittner and Kirk found that public transportation barriers have adverse effects on the populations that depend most on them for health services access, namely the poor and older persons[22]. Okoro *et al* analysed data from the 2002 Behavioral Risk Factor Surveillance System and reported finding that 9% of older adults (65 and older) did not obtain needed medical care because of transportation problems[23]. Rask *et al* reported that transportation problems were the third most common reason patients cited for not having regular medical care[24]. Transportation problems also put indirect costs on utilisation. So even availability of free medical services get affected by transportation due to financial reasons.

In a study by Maroof *et al* 2018, it was observed that out of 60, who were not utilizing health services, non-availability of companion 22 (36.7%) was the most common reason for not utilizing health services followed by health services too far 17 (28.3%), disease normal part of aging 13 (21.7%), not affordable 8 (13.3%)[14].

A study by Gnanasabai *et al*, 7.9% reported that the hospital was too far to go. The other reasons given by the 10.5% of elders were: no use of treatment, nobody to take them to the hospital, and too old to take treatment[25].

Despite perceiving transportation cost "fair or expensive," most of the respondents utilized health service (88.9%) than others. The elderly without access to healthcare personnel (71.7%) utilized health services more than those

with access to healthcare personnel (52.6%). The respondents who perceived their health status as "fair" or "poor" showed greater utilisation (85.5%) than the respondents who perceived their health status as "good" (21.7%)[11].

Availability of transport and commuting time have been found to affect also the choice of health care facility. In a Tamil Nadu study, 9% elderly switched to private facilities from government only due to lack of transport[6]. Similarly in a Dharan, Nepal study, commuting time of more than 30 minutes significantly affected choice of small private clinics over BPKIHS, Dharan – a large medical college facility ($p = .001$)[26].

Conclusion

Some enabling factors, such as financial support of family, perceived transportation cost (fair or expensive), and accessibility to healthcare personnel, were significantly associated with health service utilisation and served as significant predictors of health service utilisation[11].

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ORIGINAL ARTICLE

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A Study of Pulmonary Functions in Patients with Cirrhosis of Liver and its Correlation with the Severity of the Disease

Vignesh V, BM Singh Lamba**, Vasudha Kumari*, Nikhil Gupta***, AK Agarwal*****

Abstract

***Background:** Liver cirrhosis may develop as a terminal consequence of a wide range of diseases including alcohol-related liver disease, infections of the liver, metabolic derangements, etc. About 5 - 32% of patients with cirrhosis experience a significant vascular complication of liver disease known as hepatopulmonary syndrome (HPS). In cirrhotic individuals, HPS significantly increases the mortality and morbidity. The present study compared the pulmonary functions and the degree of arterial hypoxaemia with the severity of liver disease.*

***Methods:** The study was performedin Sharda Hospital, department of General Medicine and department of Pulmonary Medicine, School of Medical Sciences and Research, Greater Noida, Uttar Pradesh. A total of 50 patients with a confirmed diagnosis of liver cirrhosis, above the age of 18 years, were included. Patients with co-existing pulmonary diseases, heart disease, and with life-threatening complications of cirrhosis like active upper gastrointestinal haemorrhage, hepatic encephalopathy were excluded from the study.*

***Results:** PFT findings of Restrictive lung disease were seen in 14 patients and Obstructive lung disease was seen in 1 patient in total. Out of 12 patients with CTP class C, 10 patients had restrictive findings and 1 patient showed obstructive findings. Out of 17 patients in CTP class B had 4 patients with restrictive pattern. Patients with CTP class C had a higher occurrence of restrictive pattern of lung disease compared to class A and B and the difference was found to be statistically significant.*

***Conclusion:** Our study concludes that there is a significant correlation between pulmonary functions and the severity of liver cirrhosis. Patients with Child-Pugh class C when compared to patients with class A and B cirrhosis, had significantly lower PaO2, SaO2 values in ABG. Also, patients with Child-Pugh class C had lower FEV1 and FVC values in pulmonary function tests. Restrictive lung disease was more common than obstructive lung disease in patients with cirrhosis of liver.*

***Key words:** Pulmonary function test, Child-Pugh class, deranged liver function, portal hypertension.*

Introduction

The histological growth of regenerating nodules encircled by fibrous bands in response to chronic liver injury, which leads to portal hypertension and end-stage liver disease, is known as cirrhosis. Liver cirrhosis may develop as a terminal consequence of a wide range of diseases including alcohol-related liver disease, infections of the liver, toxicity, metabolic derangements, or autoimmune (AI) diseases[1].

Disease of liver leads to portal hypertension , which results from a variety of pathological conditions that increase the resistance to the portal blood flow into the liver. Due to significant structural changes brought on by fibrosis and increased vascular tone in the hepatic microcirculation, cirrhosis is primarily responsible for portal hypertension. Collateral vessel development and arterial vasodilation advance as portal hypertension worsens, increasing blood flow to the portal circulation. Eventually, the hyperdynamic circulatory syndrome develops, leading to oesophageal varices, ascites, splenomegaly, HPS and HRS[5-7].

In the absence of cardiac illness, arterial hypoxaemia frequently coexists with liver cirrhosis. Up to 45 - 50% of individuals with cirrhosis may experience impaired pulmonary function and decreased gas exchange. Generally speaking, restrictive pulmonary anomalies are seen, including an increase in the alveolar-arterial oxygen difference, airway blockage, impaired diffusion capacity, and a reduction in total lung capacity[8]. Hypoxaemia in individuals with CLD alters the course of treatment and impacts the prognosis. The link between liver illness and hypoxaemia cannot be explained by a single component alone, and its aetiology is likely to be complex[9].

About 5 - 32% of patients with cirrhosis experience a significant vascular complication of liver disease known as hepatopulmonary syndrome (HPS). In cirrhotic individuals, HPS increases mortality and may impact the incidence and seriousness of portal hypertension complications[10,11]. The age-adjusted alveolar-arterial oxygen gradient (AaPO2) is increased, there is evidence of intrapulmonary vasodilatation, and there is an indication of liver illness or

**Post-graduate Student, **Professor, ***Assistant Professor, ****Professor Emeritus, Department of Medicine, SMS and R, Sharda Hospital, Sharda University, Greater Nodia - 201 308, Uttar Pradesh.*
Corresponding Author: Dr Nikhil Gupta, Assistant Professor, Department of Medicine, SMS and R, Sharda Hospital, Sharda University, Greater Nodia - 201 308, Uttar Pradesh. Tel: 9910397098, E-mail: amon.goth666@gmail.com.

portal hypertension. If there is significant hypoxaemia, orthotopic liver transplantation may be considered a treatment for HPS.

The present study evaluated the pulmonary functions by spirometric evaluation, estimated the degree of arterial hypoxaemia by Arterial Blood Gas analysis, and correlated the pulmonary functions with the Child-Pugh score.

Materials and methods

The study was performed at Sharda Hospital, Department of General Medicine and Department of Pulmonary Medicine, School of Medical Sciences and Research, Greater Noida. The patients attending Outpatient Department (OPD) and those admitted were considered for the study. Total study participants were 50.

Inclusion criteria

Patients with a confirmed diagnosis of liver cirrhosis and above the age of 18 years.

Exclusion criteria

1. Patients with co-existing diseases like COPD, bronchial asthma, ILD.
2. Patients with heart diseases like CAD, CHF.
3. Patients with life-threatening complications of cirrhosis like active upper gastrointestinal haemorrhage, hepatic encephalopathy, and HRS.
4. Patients with a history of smoking.

Sample size: There were 50 cases for the study who will be similar to each other in terms of age, sex, and other demographic terms.

Study design

- Observational
- Cross-sectional in nature

Study duration

This was a one-and-half-year study starting from January 2021 to June 30, 2022.

Study conduct

A total of 50 cases were taken for the study. An informed consent was taken from all the subjects included in the study. A thorough history was taken and clinical examination was done. As per protocol, CBC was sent for all patients being admitted along with relevant investigations. Arterial blood gas analysis was done by a single radial puncture under local anaesthesia, while the patient was in the supine position breathing room air, breathing 100% oxygen. Arterial blood gas was analysed using ABL 800 blood analyser. The ABL 800 FLEX blood gas analyzer allows you to measure a full panel of up to 18 STAT parameters on the same blood sample. This supports fast diagnosis of critically ill patients and reduces the risks and patient discomfort associated with repeated sampling.

- pH (7.35 - 7.45)
- PaO2 (75 - 100 mmHg)
- PaCO2 (35 - 45 mmHg)
- HCO3 (22 - 26 meq/L)
- Base excess/deficit (-4 to +2)
- SaO2 (95 - 100%)

All patients were subjected to pulmonary function tests using EASYPRO (spirometer) in the standing position according to standard procedures. Forced expired volume in one second (FEV1) and forced vital capacity (FVC) were measured. Predicted values for each of the parameters were obtained from standardised references.

Method of measurement of outcome of interest

Standard statistical methods were used to measure the outcome.

Statistical analysis

Master chart of data from entire study population was created using their clinical and laboratory records. All the data obtained was analysed statistically using software like Microsoft Excel and IBM SPSS V20. Appropriate standard statistical analysis methods were used to determine factors associated with the outcome. Graphs, tables and flowcharts were used wherever appropriate. A 'p' value of < 0.05 was considered significant.

Results

The study consisted of a total of 50 subjects in which 39 were male and 11 were female.

21 subjects were CTP class A with 18 males and 3 females. 17 were CTP class B, 13 males and 4 females. 12 subjects were CTP class C, 8 males and 4 females. The distribution of subjects based on gender in CTP classes A, B, and C did not differ significantly.

The average age of patients with CTP class A was 40.86 ± 7.8 years, class B was 52.94 ± 11.52 years and class C was 52.42 ± 15.74 years.

The mean ages in CTP classes A, B, and C did not differ significantly (p value 0.052).

Table I: Distribution of study population according to gender.

	Child-Turcotte-Pugh class			Total
Gender	**A**	**B**	**C**	
Male	18	13	8	39
	85.7%	76.5%	66.7%	78.0%
Female	3	4	4	11
	14.3%	23.5%	33.3%	22.0%

p value = 0.438.

Table II: Distribution of the study participants according to age.

	Age (years)			
	Mean	**Std. Deviation**	**F-value**	**p-value**
A	40.86	7.80	2.383	0.052
B	53.94	11.52		
C	52.42	15.74		

Out of 50 subjects in the study, a total of 35 subjects had normal PFT across all the CTP classes, whereas 15 subjects showed abnormal PFT. Of these 15 subjects, 14 showed a pattern of restrictive lung disease whereas 1 showed obstructive lung disease.

Upon further comparison we found that all patients with CTP class A had a normal PFT. Out of 17 patients with CTP Class B, 4 patients had PFT suggestive of restrictive lung disease while the rest were normal. And out of 12 patients with CTP Class C, 10 patients had PFT findings suggestive of restrictive lung disease while 1 patient showed obstructive lung disease.

Patients with CTP class C had a higher occurrence of lung disease compared to class A and B and the difference was significant. Restrictive lung disease was more common than obstructive lung disease (p value 0.001).

Table III: Distribution of study population according to PFT and its correlation with CTP class A, B and C.

PFT findings	**Child-Turcotte-Pugh class**			**Total**
	A	**B**	**C**	
Normal	21	13	1	35
	100.0%	76.5%	8.3%	70.0%
Obstructive lung disease	0	0	1	1
	0.0%	0.0%	8.3%	2.0%
Restrictive lung disease	0	4	10	14
	0.0%	23.5%	83.3%	28.0%

*p value = 0.001**

The mean FEV1 (% of predicted) in subjects with CTP class C disease was 68.92 ± 7.09% whereas patients with CTP class A and B had FEV1 of 77.43 ± 5.16% and 70.88 ± 7.08% respectively. The mean FEV1 was lower in Child-Turcotte-Pugh class C compared to class A and B and the difference was found to be significant (p = 0.001).

The mean FVC (% of predicted) in subjects with CTP class C disease was 76.58 ± 8.34% whereas patients with CTP class A and B had FEV1 of 86.43 ± 2.17% and 83.47 ± 5.11% respectively. The Mean FVC was lower in Child-Turcotte-Pugh class C compared to class A and B and the difference was found to be significant (p = 0.001).

Table IV: Distribution of study population according to FEV1 and FVC and its correlation with CTP class A, B, and C.

	CTP class	**Mean**	**Std. Deviation**	**F value**	**p value**
FEV1 (% predicted)	A	77.43	5.16	8.551	0.001*
	B	70.88	7.08		
	C	68.92	7.09		
FVC (% predicted)	A	86.43	2.71	13.152	0.001*
	B	83.47	5.11		
	C	76.58	8.34		

The mean partial pressure of oxygen (PaO2) in subjects with CTP class C disease was 71.42 ± 6.53 mmHg whereas patients with CTP class A and B had PaO2 of 86.29 ± 4.16 mmHg and 81.88 ± 5.89 mmHg, respectively. The mean PaO2 was lower in Child-Turcotte-Pugh class C compared to class A and B and the difference was found to be significant (p = 0.001).

The mean arterial oxygen saturation (SaO2) in subjects with CTP class C disease was 86.17 ± 3.66% whereas patients with CTP class A and B had SaO2 of 95.86 ± 2.22% and 93.59 ± 4.47%, respectively. The mean SaO2 was lower in Child-Turcotte-Pugh class C compared to class A and B and the difference was found to be significant (p = 0.001).

Table V: Distribution of study population according to partial pressure of arterial oxygen (PAO2) and arterial oxygen saturation (SAO2) and its correlation with Child-Pugh score A, B and C.

	CTP Class	**Mean**	**Std. Deviation**	**F-value**	**p-value**
PaO2 (mmHg)	A	86.29	4.16	29.133	0.001*
	B	81.88	5.89		
	C	71.42	6.53		
SaO2 (%)	A	95.86	2.22	30.488	0.001*
	B	93.59	4.47		
	C	86.17	3.66		

Out of the total 50 subjects who participated in the study 4 gave history of platypnoea of the 4 patients who had history of platypnoea 3 had CTP class C while 1 had CTP class B disease (Table VI).

Table VI: Distribution of study population according to platypnoea.

Platypnoea	Child-Turcotte-Pugh class			Total
	A	B	C	
No	21	16	9	46
	100.0%	94.1%	75.0%	92.0%
Yes	0	1	3	4
	0.0%	5.9%	25.0%	8.0%

*p value = 0.036**

On examination of X-ray, 10 patients out of 50 had findings suggestive of pleural effusion. Of these, 9 patients had CTP class C while 1 had CTP class B disease. None of the patients with CTP class A had evidence of pleural effusion on chest X-ray.

Table VII: Distribution of study population according to chest X-ray findings.

Chest X-ray	Child-Turcotte-Pugh class			Total
	A	B	C	
Normal	21	16	3	40
	100.0%	94.1%	25.0%	80.0%
Pleural effusions	0	1	9	10
	0.0%	5.9%	75.0%	20.0%

*p value = 0.001**

Discussion

In the absence of cardiac illness, arterial hypoxaemia frequently co-exists with liver cirrhosis[56]. Additionally, up to 45 - 50% of individuals may experience changes in pulmonary function and decreased gas exchange[12]. A significant vascular consequence of liver cirrhosis illness called hepatopulmonary syndrome (HPS) affects 5 - 32% of cirrhotic individuals[16]. There is disagreement over which parameters are more significant[58], despite the fact that proposed causes of hypoxaemia include a ventilation-perfusion imbalance, an intra- or extra-pulmonary shunt, and alveolar capillary diffusion restriction.

In our study, which was carried-out at the department of General Medicine and department of Respiratory Medicine at Sharda hospital, we evaluated a total of 50 subjects of liver cirrhosis and their PFT findings. Of the 50 total subjects 39 were male and 11 were female. Our study population was further divided into 3 categories based on the Child-Turcotte-Pugh score. We had 21 subjects who were in CTP class A with 18 males and 3 females. 17 were in CTP class B, 13 males and 4 females. 12 subjects were in CTP class C, 8 males and 4 females. The distribution of subjects based on gender in CTP classes A, B, and C did not differ significantly (p value 0.438).

In our study population, the average age of subjects with CTP class A was 40.86 ± 7.8 years, class B was 52.94 ± 11.52 years and class C was 52.42 ± 15.74 years. CTP classes A, B, and C did not significantly differ in mean age in our study (p = 0.052). This was similar to other studies that were reviewed[22,23].

When we compared the pulmonary functions of our study population to the severity of liver disease we found that a total of 15 subjects showed abnormal pulmonary function. Of these 15, 14 showed a pattern of restrictive lung disease whereas 1 showed obstructive lung disease on the PFT. Restrictive lung disease was significantly more than obstructive lung disease. Patients with CTP class C had a higher occurrence of lung disease compared to class A and B and the difference was significant. Restrictive lung disease was more common than obstructive lung disease (p value 0.001). In a similar study conducted by Chaitra *et al*[16] it was found that the most common abnormality in pulmonary function tests in patients with cirrhosis of liver was restriction (35%) and only 7% had obstructive changes. The severity of hypoxaemia also correlated positively with the severity of liver disease assessed by CTP score.

In another study by Awad *et al*[15] it was found that the restrictive ventilatory function predominates. In this study, restricted ventilation affected 23 patients (46%) of all patients, obstructive ventilation affected 5 patients (10%), and combined obstructive and restrictive ventilation affected 16 patients (32%) of all patients.

The findings of the current study are also consistent with a study by Hourani *et al*[11] that looked at pulmonary dysfunction in advanced liver disease and found that 25% of patients had ventilatory limitation while only 3% had airflow obstruction. In 35% of patients, a restrictive ventilatory defect was present in addition to a diffusion anomaly. About 27% of individuals had an unusually low FEF (25 - 75%). The study also showed that numerous other causes, such as pleural effusion, ascites, or abnormalities of the interstitial lungs, were the cause of limitation in addition to the low incidence of significant abnormalities in maximal static respiratory pressure.

In the current study, FEV1 and FVC was significantly decreased among Child-Turcotte-Pugh score C compared to score A and B. The mean FEV1 in Child-Turcotte-Pugh class C was 68.92 ± 7.09% whereas patients with CTP class A and B had FEV1 of 77.43 ± 5.16% and 70.88 ± 7.08% respectively. When compared, the difference was found to

be significant among the three classes (p = 0.001).

The mean FVC in Child-Turcotte-Pugh class C was 76.58 ± 8.34% whereas patients with CTP class A and B had FEV1 of 86.43 ± 2.17% and 83.47 ± 5.11% respectively. The FVC was significantly reduced in patients with CTP class C when compared to CTP class A and B (p = 0.001). In a study conducted by Chaitra *et al*[16], it was observed that FEV and FVC values in the Child-turcotte-pugh C group were found to be lower than those in the Child-pugh A and B group. With increasing degree of hepatic dysfunction, the severity of hypoxaemia also rose proportionately. In another study, Awad *et al*[15] found that in group III (child C) patients forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were substantially lower than those in group II (I and II). Additionally, when group (I) and group (II) were examined, forced expiratory flow at 25% to 75% and forced vital capacity (FEF 25 - 75%) revealed a statistically significant difference (II and III).

In our study, we found that the mean partial pressure of oxygen (PaO2) in subjects with CTP class C disease was 71.42 ± 6.53 mmHg whereas patients with CTP class A and B had PaO2 of 86.29 ± 4.16 mmHg and 81.88 ± 5.89 mmHg, respectively. We also found that the mean arterial oxygen saturation (SaO2) in subjects with CTP class C disease was 86.17 ± 3.66% whereas patients with CTP class A and B had SaO2 of 95.86 ± 2.22% and 93.59 ± 4.47%, respectively. The difference among the three classes for both PaO2 and SaO2 was significant. The findings of our investigation were in agreement with those of Medha *et al*[22], who investigated arterial hypoxaemia in patients with liver cirrhosis and found that patients with chronic liver disease and cirrhosis had lower arterial oxygen tension and oxygen saturation. Awad *et al*[15] also reported that the mean partial arterial carbon dioxide pressure (PaCO2) in group III (Child C) was significantly lower when compared to group I (Child A) and group II (Child B). In the study conducted by Chaitra *et al*[16], it was observed that there was progressive decrease in PaO2 and SaO2 values as the child-pugh score increased. Konstantinos *et al*[8] discovered that decreased PaO2 and SaO2 levels were closely connected to advanced liver cirrhosis and higher grades of ascites.

In our study 4 patients gave a history of platypnoea. Out of the 4 patients 3 had CTP class C while 1 had CTP class B disease. Anand *et al*[28] in his study at Haryana reported significantly higher incidence of dyspnoea, platypnoea in patients with HPS.

In our study, on examination of X-ray, 10 patients out of 50 had findings suggestive of pleural effusion. Of these, 9 patients had CTP class C while 1 had CTP class B disease. None of the patients with CTP class A had evidence of pleural effusion on chest X-ray. This difference was statistically significant. In the study conducted by Awad *et al*[15], it was found that pleural effusion was the commonest clinical finding in examination of the respiratory system of 11 (18.3%) patients which was followed by consolidation in (3.4%) patients.

Conclusion

Our study concludes that derangements in pulmonary functions are seen in patients with cirrhosis of the liver. PFT findings of restrictive lung disease were more frequent than obstructive lung disease. Also severity of cirrhosis correlates directly with the incidence of lung disease. In our study, patients with CTP class C cirrhosis had significantly deranged pulmonary function when compared to patients with CTP class A and B.

We also found that patients with CTP class C cirrhosis had significantly lower PaO2, SaO2 values in ABG when compared to CTP class A and B. Also, patients with child-pugh class C had lower FEV1 and FVC values in pulmonary function tests. Significant decreases in PaO2, SaO2, FEV1 and FVC are associated with restrictive pulmonary function patterns in patients with severe liver cirrhosis and ascites . These Patients are more susceptible to infection and adult respiratory distress syndrome (ARDS) as a result of reduced pulmonary resistance. Accordingly, depending on combined hepatic and pulmonary disorders, the prognosis for those individuals is poor.

Based on the findings of our study we recommend that a patient with cirrhosis of the liver should also be evaluated for pulmonary function with pulmonary function tests and arterial blood gas analysis. This would help to quantify the compromise in the pulmonary function so that remedial measures may be taken which would help to prevent pulmonary complications in such patients.

Limitations: This study has a small sample size. It is a single-centre study. Being a hospital-based study, there is a bias factor in selection of subjects. Only symptomatic patients who presented to hospital were studied.

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MEDICAL COUNCIL OF INDIA (MCI)/NATIONAL MEDICAL COMMISSION (NMC) GUIDELINES FOR AUTHORS (AMENDED), 2020

As per notification No. MCI-12(2)/2019-Med. Misc./189334 dated 12 February, 2020 published in Extraordinary Gazette of Govt. of India, the MCI/NMC has made changes to amend the "Minimum Qualifications for Teachers in Medical Institutions Regulations, 1998". These will be part of "Minimum Qualifications for Teachers in Medical Institutions (Amendment) Regulations, 2019" and shall come into force from the date of their publication in the Official Gazette.

1. Original papers, meta-analysis, systematic reviews, and case series that are published in journals included in Medline, Pubmed Central, Citation index, Sciences Citation index, Expanded Embase, Scopus, Directory of Open access journals (DoAJ) will be considered.
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ORIGINAL ARTICLE

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Relationship between Peripheral Neuropathy and Carotid Intima-Media Thickness in Type 2 Diabetes Mellitus Patients

Manoj Saluja, Sidharth Sharma**, Prakarsh Sharma**, Saurabh Chittora***, Deelip Meena**, Gaurav Bhargava**, Drishya Pillayi**, Komal V Saluja*****

Abstract

Introduction: Diabetic polyneuropathy (DPN) is the most common diabetic complication and has a lifetime prevalence of approximately 50%. Diabetic peripheral neuropathy is the leading cause of disability due to foot ulceration, amputation, and gait disturbance. Almost 20% to 30% of patients with diabetic peripheral neuropathy suffer from neuropathic pain, which significantly lowers the quality of life and dramatically increases health costs associated with diabetes. Carotid intima-media thickness (CIMT), assessed using B-mode ultrasound, has already established itself as a surrogate endpoint in monitoring subclinical atherosclerosis.

Methods: This cross-sectional study includes 200 diabetic patients who attended the diabetic clinic in Government Medical College, Kota, in 2022. CIMT was measured in the bilateral proximal internal carotid artery and mid-common carotid artery using B-mode ultrasonography. Using a vibrosense biothesiometer, the vibration perception threshold (VPT) was assessed.

Results: This study evaluated 200 diabetic patients (107 males and 93 females). Of these patients, 112 had peripheral neuropathy. Patients with peripheral neuropathy were older (65.6 ±12.9 years vs 64.1 ±13.5 years) and had a longer duration of T2DM (10.2 ± 4.8 years vs 8.3 ±4.9 years) than patients without peripheral neuropathy, p-value < 0.05. Patients with CIMT >0.8 mm had higher prevalence of peripheral neuropathy (63.4% vs 31.9% in patients with CIMT < 0.8 mm). In these patients, prevalence of hypertension was higher (82.3% vs 63.8% in patients with CIMT < 0.8 mm).

Conclusion: This study revaled a significant relationship between peripheral neuropathy and CIMT in patients of Type 2 diabetes mellitus.

Key words: Diabetes mellitus, DPN, CIMT, VPT.

Introduction

Human behaviour and lifestyle changes over the last century have resulted in a remarkable increase in the global prevalence of diabetes. The epidemic is mostly of type 2 diabetes mellitus and associbated conditions like "diabesity" and "metabolic syndrome"[1,2].

Diabetic polyneuropathy (DPN) is the most common diabetic complication and has a lifetime prevalence of approximately 50%. DPN is the leading cause of disability due to foot ulceration, amputation, and gait disturbance. Almost 20% to 30% of patients with DPN suffer from neuropathic pain, which significantly lowers the quality of life and dramatically increases health costs associated with diabetes[3]. Epidemiological studies show that type 2 diabetes has a greater prevalence of diabetic neuropathy than type 1 diabetes. Undiagnosed DPN complications lead to impaired quality of life and an increase in mortality rate. Vibration perception threshold (VPT) plays an essential role in the early detection of DPN and consequently reduces its complications.

Metabolic syndrome is a cluster of dangerous cardiovascular risk factors: central obesity, glucose intolerance, hypertension, and dyslipidaemia. People with metabolic syndrome are at higher-risk for cardiovascular disease and increased mortality. Recently, many studies have suggested that the components of the metabolic syndrome had a prominent role in the pathogenesis of peripheral neuropathy. It is generally believed that oxidative stress is the primary pathological mechanism causing nerve damage in diabetes. Oxidative stress is possibly triggered by vascular abnormalities and associated microangiopathy in the nerve. It is a crucial pathological process inducing nerve damage in diabetes in humans and experimental models[4].

DPN is a common microvascular complication with high mortality rates, but little is known about the association between DPN and atherosclerotic vascular changes. CIMT, assessed using B-mode ultrasound, has already established itself as a surrogate end-point in monitoring subclinical atherosclerosis. Recent studies have demonstrated the relationship between peripheral neuropathy and atherosclerotic vascular changes[5,6]. Furthermore, these

**Senior Professor and Unit Head, **Senior Resident, ***Assistant Professor, ****Medical Student, Department of Medicine, Government Medical College, Kota - 324 005, Rajasthan.*

Corresponding Author: Dr Sidharth Sharma, Senior Resident, Department of Medicine, Government Medical College, Kota - 324 005, Rajasthan. Tel: 8502968208, E-mail: sidharthsharmambbs@gmail.com.

studies consistently showed a relationship between functional parameters of arterial stiffness like pulse wave velocity and peripheral neuropathy. However, results regarding the relationship between CIMT and peripheral neuropathy are contradictory[5,6].

The present study explored the relationship between peripheral neuropathy and CIMT in type 2 diabetes mellitus patients.

Material and Methods

This cross-sectional study included 200 patients with established and newly diagnosed cases of type 2 diabetes mellitus of both sexes, aged between 21 to 80 years, who attended the diabetic clinic at Government Medical College, Kota, from January 2022 to April 2022. Patients with a history of alcoholism and smoking were excluded, and those with a history of ischemic heart disease and chronic kidney disease were also excluded from the study. Following an explanation of the nature and purpose of the study, subjects willing to participate were included. Ethics committee clearance and informed consent were obtained. The diagnosis of diabetes was made as per American Diabetes Association criteria. An elaborative history, including duration, co-morbidities and drug compliance, was taken, and a thorough clinical examination was performed on each participant. Anthropometric measurements of height, weight, body mass index, and waist circumference were taken. CIMT was measured in the bilateral proximal internal carotid artery and mid-common carotid artery using B-mode ultrasonography. CIMT value > 0.8 mm was considered as increased thickness[7]. Using a Vibrosense biothesiometer, the vibration perception threshold (VPT) was assessed, and a VPT value of > 25 V was taken as the standard for diagnosing peripheral neuropathy[8]. A venous blood sample was drawn and HbA1c, fasting blood sugar, fasting insulin, and lipid profile were measured.

Statistical methodology

Statistical analysis was performed using Statistical Package for Social Science (SPSS) Version 22.0. Quantitative Continuous variables data were expressed as mean ± standard deviation, whereas Quantitative discrete variables data were expressed as frequencies and numbers (%). The qualitative data were expressed in Medians with interquartile ranges. The student's t-test and χ2test were used to compare the difference for means between two or more groups or categorical variables. In contrast, continuous variables were compared using the Mann- Whitney U test. All statistical tests were two-tailed. The Pearson correlation co-efficient was computed to determine the association between 2 continuous variables. Statistical significance was taken as $p < 0.05$.

Results

This study evaluated 200 diabetic patients (107 males and 93 females). Of these patients, 112 had peripheral neuropathy. Patients with peripheral neuropathy were older (65.6 ± 12.9 years vs 64.1 ± 13.5 years) and had a longer duration of type 2 diabetes mellitus (10.2 ± 4.8 years vs 8.3 ± 4.9 years, P-value < 0.05). The prevalence of hypertension was higher in patients with peripheral neuropathy than in those without peripheral neuropathy. In addition, fasting blood sugar, fasting insulin, and triglycerides were higher in patients with peripheral neuropathy, but the results were not statistically significant (Table I and Table II).

Table I: Baseline characteristics.

Clinicopathologic Factors		Group (N=200)
Gender	Male	107 (53.5%)
	Female	93 (46.5%)
Age (years)	Mean ± SD	64.92 ± 13.12
	Range	(22 - 99)
Duration of diabetes (years)	Mean ± SD	9.4 ± 4.9
	Range	(0 - 32)
Hypertension	Yes	156 (78%)
	No	44 (22%)
Height (mt)	Mean ± SD	1.63 ± 0.08)
	Range	(1.41 - 1.84)
Weight (kg)	Mean ± SD	66.72 ± 11.65
	Range	(42 - 98)
BMI (kg/m^2)	Mean ± SD	25.09 ± 4.8
	Range	(15.9 - 39.6)
Waist Circumference (cms)	Mean ± SD	97.74 ± 7.84
	Range	(72 - 122)
FBS (mg/dl)	Mean ± SD	155.9 ± 68.6
	Range	(81 - 545)
HbA1c (%)	Mean ± SD	7.42 ± 1.53
	Range	(4 - 13.6)
Fasting Insulin (μlU/ml)	Mean ± SD	18.52 ± 16.15
	Range	(1.11 - 97.1)
TG (mg/dl)	Mean ± SD	178.98 ± 60.8
	Range	(58 - 482)
HDL (mg/dl)	Mean ± SD	44.26 ± 10.07
	Range	(28 - 81)
Cholesterol (mg/dl)	Mean ± SD	186 ± 16.28
	Range	(125 - 225)
NCV	Mean ± SD	26.76 ± 6.9
	Range	(13.95 - 52.58)
VPT	Mild	27 (13.5%)
	Moderate	61 (30.5%)
	Severe	112 (56%)
CIMT (mm)	Mean ± SD	0.86 ± 0.16
	Range	(0.40 - 2.43)
Metabolic Syndrome	Yes	155 (77.5%)
	No	45 (22.5%)

Table II: Comparative analysis of patients based on peripheral neuropathy.

Clinicopathologic Factors		Peripheral neuropathy (-) (n = 88)	Peripheral neuropathy (+) (n = 112)	F value/Chi square test (P value)	Remark
Gender	Male	53 (60.3%)	54 (48.2%)	2.859 (0.090)	Not Significant
	Female	35(39.8%)	58 (51.8%)		
Age (Years)	Mean ± SD	64.1 ± 13.5	65.6 ± 12.9	0.4361 (0.6088)	Not Significant
	Range	(22 - 92)	(33 - 99)		
Duration (Years)	Mean ± SD	8.3 ± 4.9	10.2 ± 4.8	**6.9245 (0.0091)**	**Significant**
	Range	(0 - 32)	(0 - 30)		
Hypertension	Yes	58 (65.9%)	98 (87.5%)	**13.387 (0.0002)**	**Significant**
	No	30(34.1%)	14 (12.5%)		
Height (mt)	Mean ± SD	1.63 ± 0.08	1.64 ± 0.08	0.7694 (0.3814)	Not Significant
	Range	(1.45 - 1.84)	(1.41 - 1.84)		
Weight (kg)	Mean ± SD	67.5 ± 13.1	66.1 ± 10.4	0.7028 (0.4028)	Not Significant
	Range	(42 - 98)	(45 - 96)		
BMI (Kg/m^2)	Mean ± SD	25.51 ± 5.2	24.78 ± 4.5	1.149 (0.284)	Not Significant
	Range	(16.7 - 39.6)	(15.9 - 36.8)		
Waist Circumference (cms)	Mean ± SD	97.8 ± 7.9	97.7 ± .7.8	0.03199 (0.8582)	Not Significant
	Range	(78 - 122)	(72 - 115)		
FBS (mg/dl)	Mean ± SD	150.8 ± 55.5	159.9 ± 77.3	0.8669 (0.3529)	Not Significant
	Range	(85 - 362)	(81 - 345)		
HbA1c (%)	Mean ± SD	7.34 ± 1.48	7.49 ± 1.58	0.4780 (0.4901)	Not Significant
	Range	(4 - 11.6)	(4.8 - 13.6)		
Fasting Insulin (μIU/ml)	Mean ± SD	17.15 ± 14.2	19.59 ± 17.52	1.1303 (0.2889)	Not Significant
	Range	(1.28 - 69.6)	(2.30 - 97.1)		
TG (mg/dl)	Mean ± SD	173.4 ± 49.9	183.3 ± 68.1	1.3049 (0.2546)	Not Significant
	Range	(91 - 412)	(58 - 482)		
HDL (mg/dl)	Mean ± SD	43.5 ± 9.8	44.9 ± 10.3	0.9055 (0.3424)	Not Significant
	Range	(30 - 81)	(28 - 72)		
Cholesterol (mg/dl)	Mean ± SD	185.4 ± 18.9	186.4 ± 13.9	0.2142 (0.6439)	Not Significant
	Range	(125 - 225)	(154 - 225)		
VPT (v)	Mean ± SD	21.6 ± 2.80	30.85 ± 6.38	**162.06 (0.000)**	**Significant**
	Range	(13.95 - 24.96)	(25.07 - 52.58)		
CIMT (mm)	Mean ± SD	0.82 ± 0.12	0.90 ± 0.17	**12.78 (0.0004)**	**Significant**
	Range	(0.40 - 1.18)	(0.6 - 2.43)		
Metabolic Syndrome	Yes	64 (72.7%)	91 (81.3%)	2.053 (0.151)	Not Significant
	No	24 (27.3%)	21 (18.8%)		

Table II shows that the CIMT value was higher among peripheral neuropathy subjects (0.90 ± 0.17 mm vs 0.82 ± 0.12 mm) than among patients without neuropathy, (p-value < 0.05). In our study, out of 200 patients, 155 patients had metabolic syndrome. The peripheral neuropathy group had more patients with metabolic syndrome (81.3% of patients with peripheral neuropathy had metabolic syndrome vs 72.7% of patients without peripheral neuropathy had metabolic syndrome, but the results were not significant).

Table III shows that patients with CIMT > 0.8 mm had a higher prevalence of peripheral neuropathy (63.4% vs 31.9% in patients with CIMT < 0.8 mm). In these patients prevalence of hypertension was higher (82.3% vs 63.8% in patients with CIMT < 0.8 mm). Patients with CIMT > 0.8 mm had a longer duration of diabetes (10.9 ± 4.13 years vs 4.96 ± 4.36 years), and more patients had metabolic syndrome (81.6% vs 66.7%); all these results were statistically significant.

Table III:

Clinicopathologic Factors		CIMT< 0.8 mm (n = 47)	CIMT ≥ 0.8 mm (n = 153)	Chi square test	(p value)	Remark
Peripheral neuropathy (VPT)	Negative	32 (68.1%)	56 (4.6%)	14.464	**0.0001**	**Significant**
	Positive	15(31.9%)	97 (63.4%)			
Hypertension	Yes	30(63.8%)	126 (82.3%)	7.1891	**0.0073**	**Significant**
	No	17(26.2%)	27 (17.7%)			
Metabolic Syndrome	Yes	31 (66.7%)	124 (81.6%)	4.6941	**0.0302**	**Significant**
	No	16 (33.3%)	29 (19.4%)			
Duration of diabetes (years)	Mean ± SD	4.96 ±4.36	10.9 ± 4.13	77.316	**<0.0001**	**Significant**
	Range	(0-17)	(4 - 32)			

Table IV shows that VPT positively correlated with duration of diabetes (r value = 0.242), fasting blood sugar (r value = 0.194), fasting insulin (r value = 0.146) and CIMT (r value = 0.244).

Table IV: Pearson correlation of different variables with VPT.

Parameters	R value (Pearson correlation co-efficient)	P value	Results
Age (in yrs)	-0.048	0.501	Not significant
Duration of diabetes	0.242	**0.001**	**Significant**
Height	0.047	0.504	Not significant
Weight	-0.50	0.484	Not significant
BMI	-0.66	0.353	Not significant
Waist Circumference	-0.20	0.777	Not significant
FBS	0.194	**0.006**	**Significant**
HbA1c	0.117	0.099	Not significant
Fasting Insulin	0.146	**0.039**	**Significant**
Triglycerides	0.051	0.471	Not significant
HDL	0.054	0.447	Not significant
Total Cholestrol	0.086	0.225	Not significant
CIMT	0.244	**0.001**	**Significant**

Discussion

Cardiovascular disease is a major complication of type 2 diabetes, and atherosclerosis is usually asymptomatic in these patients. Atherosclerosis is a multifactorial disease, frequently involving the entire arterial system. Thus, the severity of atherosclerotic change in any segment of the arterial system can provide information about involvement in other arterial systems. Therefore, determining the extent of atherosclerosis in carotid arteries, that are easily visualised, may provide information about the presence and severity of coronary atherosclerosis.

Increased CIMT, an early marker of atherosclerosis, was significantly higher in the peripheral neuropathy group than in the group without peripheral neuropathy. Many previous studies found a relationship between peripheral neuropathy and functional parameters of arterial stiffness like brachial-ankle pulse-wave velocity (PWV) and brachial pulse pressure (PP)[9]. In the present study, we also demonstrated a positive correlation between CIMT (an early atherosclerosis marker) and VPT. Patients with CIMT > 0.8 mm had more peripheral neuropathy than those with CIMT < 0.8 mm. A previous study demonstrated that CIMT was higher in patients with versus without peripheral neuropathy, which is one of the chronic complications of diabetes[5]. It has been found that intima-media thickening, an early sign of atherosclerosis, is associated with cardiovascular risk factors. Many studies suggest that increased CIMT is associated with an increased risk of stroke and silent cerebral infarction[10]. Patients with peripheral neuropathy were older and had a longer duration of diabetes. In addition, patients with severe peripheral neuropathy had a higher prevalence of hypertension. Forrest *et al*, found that hypertensive individuals had a significantly increased risk of developing DPN in a 6-year follow-up study[11]. There was also a significant positive correlation between peripheral neuropathy and fasting blood glucose and insulin. Dyck *et al* found a strong correlation between hyperglycaemia and peripheral neuropathy[12]. Many studies demonstrated a relationship between age and duration of diabetes with peripheral neuropathy, similar to our study[13,14].

Metabolic syndrome is also associated with increased CIMT. In the present study, CIMT values were higher (> 0.8 mm) among the patients of metabolic syndrome compared to patients without metabolic syndrome, and the results were statistically significant. Similar findings were observed in other studies in which metabolic syndrome was associated with increased CIMT progression[15,16].

It is generally believed that oxidative stress is the primary pathological process inducing nerve damage in diabetes. Oxidative stress, possibly triggered by vascular abnormalities and associated microangiopathy in the

nerve, is a crucial pathological process causing nerve damage in diabetes in humans and experimental models. Other possible mechanisms include decreased Na+/K+ ATPase activity, increased vasoconstrictors such as thromboxane A2 and endothelin levels, and decreased vasodilators such as prostaglandin I2 and nitric oxide, increased aldose reductase activity, and fatty deposition in nerves, extracellular protein glycation, mitochondrial dysfunction[2,4].

Limitations: It was a cross-sectional design, thus it was not possible to establish a cause-and-effect relationship between peripheral neuropathy and CIMT. Further, small sample size of the study population also limits the generalisation of the results.

Conclusion

This study determined a significant relationship between peripheral neuropathy and CIMT in patients with type 2 diaetes mellitus. Early detection of peripheral neuropathy might help prevent macrovascular complications of diabetes. Furthermore, evolving data suggest that exercise and weight reduction strategies are helpful in patients with peripheral neuropathy in the setting of diabetes and metabolic syndrome, along with glycaemic control. Thus, implementing strategies that target these modifiable risk factors can help prevent and control the chronic complications of type 2 diabetes mellitus.

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REVIEW ARTICLE

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Tuberculin Test: A Reappraisal for the Modern Internist

Rudrajit Paul, Kumkum Bhattacharya**, Dipanjan Bandyopadhyay****

Abstract

As India is aiming for Tuberculosis elimination, there is an urgent need for strengthening both the diagnostic and therapeutic aspects of the disease. The Tuberculin test is an age-old diagnostic method for tuberculosis infection, both active and latent. But interpretation of the test is still mired in controversy and clinicians often fail to agree on the significance of the test. This article is aimed at resolving some of the controversy surrounding the test and presenting current evidence on its proper use. Interpretation of the test in different clinical settings (like after BCG vaccination and in HIV positive persons) has been discussed. The molecular basis of the test has also been touched upon. A comparison with the IGRA test has been presented. Lastly, rationale for use of the test in the Indian setting has been discussed by the authors.

Key words: Mantoux test, Latent TB, PPD, induration, BCG vaccine.

Introduction

Mycobacterium tuberculosis infection remains one of the main public health problems in developing countries like India. The World Tuberculosis Report, 2022 released by the WHO, shows that India is the country with the highest tuberculosis (TB) burden in the world and the country with the highest proportion of TB deaths[1]. Parallel to the high incidence of TB in the country, another caveat is the rise of multi-drug resistant TB infections[1]. However, in India, a large number of TB patients are still undiagnosed[2]. Out of the projected number of new TB patients in the country, only about 60% are documented each year[2]. So, various operational issues including, but not limited to, the lapses in diagnostic methodology contribute to this large chunk of "missing" TB patients in the country. These patients may act as superspreaders and further propagate the silent epidemic. So, like any other epidemic, control measures must start with proper and timely diagnosis.

Thus, it is imperative that India has a robust method for early diagnosis of TB. The gold standard for diagnosis of TB is microbiological proof of the infection: direct AFB (Acid-Fast Bacilli) stain, mycobacterial culture or genetic tests. Besides this technique, the other major diagnostic process is the tuberculin test[3]. This test has considerable importance from epidemiological point of view but is often under-utilized. As subsequent discussions will show, this age-old test still has a lot of controversies surrounding it and clinicians often have hair-splitting mentality about its utility in clinical care. As India is aiming for a zero-tuberculosis future (Pradhan Mantri TB-Mukt Bharat Abhiyaan), it is imperative that a reappraisal of this time-tested diagnostic method is done and clinicians are amply informed about the nuances of the test.

History

The tuberculin was first described by Dr Robert Koch, way back in 1890[4]. It was he who first identified the tuberculosis bacillus in 1882 and in 1890, he announced the discovery of a substance called "tuberculin", which was a crude extract from the heat-killed bacterial culture. But the subsequent use of "tuberculin" was quite different from what Koch had envisaged in 1890.

Dr Koch thought that tuberculin would be like a vaccine to protect against the TB infection and he advocated serial injections to patients. Although claims about its protective role were soon debunked by his contemporary physicians, and that TB vaccine never really materialized, it was nonetheless found to be useful in diagnosis of the same infection. Soon, the works of Charles Mantoux and Clemens von Pirquet established the groundwork for using this tuberculin extract as a reagent for a skin test to detect TB[3]. This was the tuberculin skin test (TST). The basic tenets of the test have remained unchanged after that. Thus, this test is more than 100-years-old but it still has a lot of unanswered questions.

Types of tuberculin test

As stated, tuberculin test is an intradermal test that elicits a delayed type hypersensitivity reaction. More about the

Consultant Physician, Department of Medicine, Ruby General Hospital, Kolkata - 734 107, (WB); **Professor, Department of Microbiology, IPGMER and SSKM Hospital, Kolkata - 734 120, (WB); ***Professor and HOD, Department of Medicine, North Bengal Medical College, Siliguri - 734 012, (WB).

Corresponding Author: Dr Rudrajit Paul, Department of Medicine, Ruby General Hospital, Kolkata - 734 107, (WB). Tel: 9433824341, E-mail: r.paul.medicalcollege@gmail.com.

molecular basis of the test will be discussed in the next section. But first, a brief discussion on the types of tuberculin test:

1. Mantoux test: The commonest, and probably the only method used now. An intradermal injection of the reagent is given and the subsequent reaction noted (more on this later).
2. Von Pirquet test[5]: Here, the reagent is given by a cutaneous scratch. In the original description of 1909, the scratch was made with a smallpox vaccination lancet.
3. Moro test[6]: This is a skin patch test. In earlier times, a tuberculin ointment was available, which was applied on the skin. This was most suitable for children. At that time, many authors reported that the results of the test had to be read not on the third day, but sixth or seventh day.
4. Calmette test: 10% aqueous solution of tuberculin, applied to the eye, produced marked conjunctivitis (Parker HC, 1908).
5. Trambusti's test[6]: Here, a needle, dipped in 10% tuberculin solution, is thrust into the skin to a depth of 5 mm, twisted 2 - 3 times and withdrawn.
6. Hamburger test[7]: Subcutaneous injection of tuberculin.
7. Craig test[7]: Multiple puncture method, like smallpox vaccination.
8. Stewart test: Single puncture of skin. But unlike Pirquet test, this is done not with a lancet, but with a needle.
9. Tine test: Here, a small button containing 4 - 6 small needles coated with TB antigens is pressed against the skin. Interpretation is similar to Mantoux test.
10. Heaf test: This is also a multiple puncture technique where a spring-loaded gun is used. Here, the diameter of the largest papule is measured. However, this test is no longer applicable because the PPD-S that is available now has been standardised only for the Mantoux test. That solution should not be used for Heaf test. Heaf Test was mainly done with the old tuberculin solution (not available now). This was also called *Sterneedle* Test.

(Except for No. 1, all others are of historical interest only).

The basic premise of all of these tests is the same. A small dose of tuberculin, given to an infected individual, will lead to a visible cutaneous reaction. This reaction will be a marker for the diagnosis of TB infection. But infection does not mean active disease. In many cases, the organism remains latent. So, the test is more important for its negative predictive value. Absence of a reaction (if false negatives can be ruled out: see later) indicates absence of the bacilli in the body. This is important in some cases like before starting biologic therapy in rheumatology.

The reagent used for the test was first prepared by protein extraction from heat-killed cultures of *M. tuberculosis*. This extract was known as old tuberculin (OT)[4]. However, it had a lot of extraneous proteins including beef extracts from the culture. In 1930, Florence Seibert spearheaded the production of a chemically purer form of OT and called it purified protein derivative (PPD). This contained less carbohydrates than the OT and was less prone to non-specific hypersensitivity reactions[4]. Now, for Mantoux test, this PPD is used. In 1941, Seibert prepared a large batch of PPD which was designated as the standard and the USFDA ruled that all subsequent batches of PPD must have a bioassay to demonstrate equal potency to this standard PPD (PPD-S). In 1952, the WHO declared this PPD-S as the standard of quality internationally.

In 1957, the Statens Serum Institute of Copenhagen prepared a large batch of tuberculin for UNICEF. It was called PPD RT 23 (RT: Research Tuberculin)[8]. This is also considered an international standard. 5 units of PPD-S is equivalent to 2 U of PPD-RT 23. At present, both PPD-S and PPD-RT 23 are considered as accepted standards internationally[9]. Any commercially available PPD is mixed with Tween 80, a detergent added to prevent its adsorption on glass. But this does not interfere with test. The testing method along with result interpretation, as described in guidelines, is for PPD mixed with Tween 80 (0.005%).

Now, according to the European Pharmacopoeia, PPD is produced from *M. bovis* and/or *M. tuberculosis*. Production is based on the seed-lot system.

In India, this is manufactured by the BCG Vaccine Laboratory, Guindy, Chennai (Tamil Nadu). But now, other commercial units have also been licensed to produce it.

PPD from other strains of mycobacteria are also prepared (Table I), although their clinical utility is doubtful[3]. They are mainly useful for research purposes like finding the prevalence of environmental mycobacteria.

Table I: Purified protein derivative (PPD) from different mycobacteria species[3].

PPD type	Species of mycobacteria
PPD-S	*M. tuberculosis*
PPD-A	*M. avium*
PPD-G	Gause strain of scotochromogen
PPD-F	*M. fortuitum*
PPD-Y	*M. kansasii*
PPD-B	Non-photochromogen Battey bacilli (*Mycobacterium intracellulare*)

Although culture extracts are still the main source of PPD, there have been attempts to produce recombinant proteins matching *M. tuberculosis* membrane proteins[10]. However, this is still not commercially produced.

Molecular basis of the test

The tuberculin reaction, as utilised in this test, follows the pathway of delayed type hypersensitivity. This is a special type of cell-mediated immunity (CMI). Here, it would be prudent to mention that the same *M. tuberculosis* antigens cause two types of type IV reaction. One is the tuberculin-type reaction, which will be discussed next. And the second one is the granulomatous reaction, which is more severe and found at sites of the disease.

In CMI, the reaction is mainly dependent on T lymphocyte activity, and antibodies or complement have a miniscule role. When the antigen is presented by the antigen presenting cells, the Th0 cells (CD4+) transform into Th1 cells, which drive the CMI response. This transformation takes 12 - 24 hours, hence called "delayed". γ-IFN (secreted by Th1 cell) plays an important role in this pathway.

The tuberculin-type hypersensitivity involves injection of a soluble antigen into the skin with subsequent development of local and systemic (in some cases) reaction. This similar reaction has been documented with *M. tuberculosis*, *M. leprae* and also some other agents like Beryllium. So, the test is not exclusive to TB.

The TST is an example of recall response. Exposure to soluble antigens to which that person had been previously exposed leads to the following cascade of reactions (Fig. 1).

This is where the tuberculin reaction stops and in a few days, the induration also disappears. In this figure, only the local reaction has been described. But very rarely, there may also be some systemic reactions like fever (due to excess TNF-α and IL-1). But if, instead of the soluble antigen, there is live bacilli, then the reaction progresses to form granuloma. This happens because the live bacilli, engulfed by the macrophages, are not killed intracellularly. These macrophages with intracellular live bacilli form the granulomatous reaction by forming giant cells and surrounding necrosis.

Basic technique of tuberculin skin test (TST): Essential points

The proper technique of TST is very important because any deviation from the standard will lead to false results:

1. Only Tuberculin PPD solution for Mantoux test, which has been standardised, should be used for the purpose.
2. The solution is available in four different concentrations:

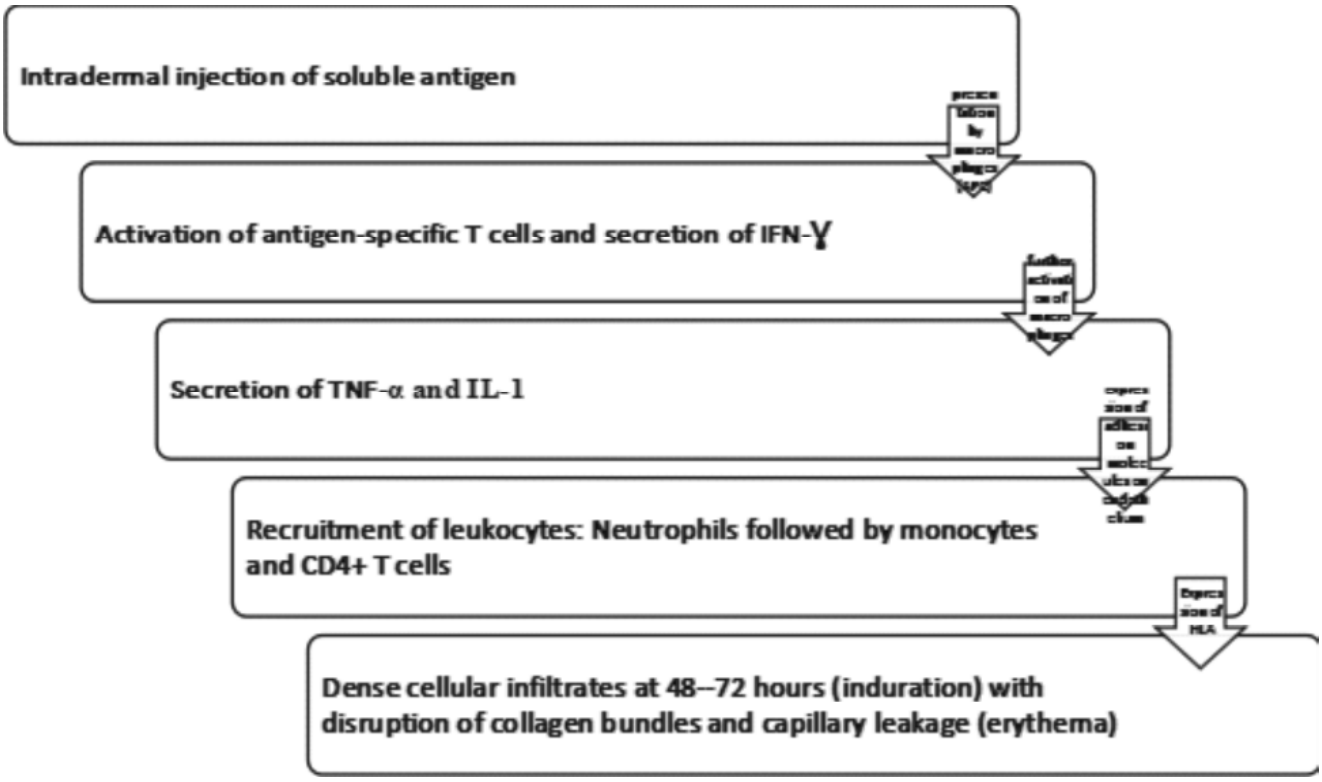

Fig. 1: *The tuberculin reaction physiology (APC: antigen presenting cell).*

1 TU/0.1 mL, 2 TU/0.1 mL, 5 TU/0.1 mL and 10 TU/0.1 mL (TU: Tuberculin unit). 1 TU = 0.02 microgram of PPD-S or PPD-RT23. This means that the volume of the solution injected intradermally will always be 0.1 mL, even if the dose of PPD varies. So, if a patient needs 5 TU of PPD, we will not inject 0.5 ml of 1 TU/0.1 mL solution but we will use the 5 TU/0.1 mL solution only.

3. The tuberculin solution must be stored at 2 - 8° C. It must never be frozen. It must never be exposed to sunlight.
4. The skin test is done on the volar surface of the non-dominant forearm, unless there are problems like local infection or scarring. That particular anatomical area has been chosen for ease of testing only. Thus, if a person has amputated hands, the TST may be done anywhere else in the body.
5. There is no need to sterilize the site before the test, but it should be clean.
6. The injection is given only with the tuberculin syringe supplied for the purpose and not any other syringe.
7. During injection, the needle (26- or 28-gauge) bevel should be facing upwards.
8. Since this is an intradermal injection, if given correctly, there should be no significant bleeding from the site.
9. If the injection technique is correct, a pale wheal of 6 - 10 mm should be raised.
10. If the wheal is not raised properly or is too small on first attempt, the injection of PPD should be repeated 2 inches away from the first site or on the other arm.
11. The patient should be instructed not to apply any chemicals to the area and not to scratch the area. Also, the area should not be covered.
12. Observation is made after 48 - 72 hours. If a patient misses this deadline, the test will be invalidated.
13. The induration at the site of injection is measured perpendicular to the long axis of the arm. Erythema is of no importance in interpretation.
14. The diameter of the induration is measured in millimetres. If there is no induration, it is recorded as "0 mm".
15. The test has not been adequately studied in elderly subjects to make any firm recommendations. But it is generally considered to be similar to young adults. But there is a point of view that in the elderly group, the reading of the Mantoux test may be done at 96 hours instead of 72 (Package insert for Aplisol®; FDA), although this is not a firm recommendation.
16. Although there are 10 TU/0.1 mL strength solutions available, their utility is limited. Some countries like Australia prefer to use the 10 TU dose for TST. But most countries like India use the 5 TU/0.1 mL PPD-S dose.
17. After becoming positive, the induration may remain for up to 1 week.

Adverse reactions of the test

1. Mild pain or pruritus may occur at the injection site for some time. This is usually self-limiting. If excessive, this may be controlled with ice packs.
2. In strongly positive reaction, an ulcer or blister may develop at the site of the test (1 - 2%)[11]. This may later form a scar rarely.
3. Rarely, regional lymphadenitis has been reported[12].
4. Wrong method of injection may lead to formation of a haematoma locally.
5. Immediate allergic reaction (within 12 hours) may develop at the injection site. This is protein allergy, but does not indicate TB infection. Usually, this is self-limiting. Anaphylaxis to the reagent has been reported extremely rarely[12].

Interpretation of TST

When will the test be positive

A Mantoux test will be positive if there is current tuberculosis infection in the body (*M. tuberculosis* or *M. bovis*). However, infection does not always mean active disease. The test cannot differentiate between active and latent infection.

What is a positive test?

A reaction to the tuberculin test, as indicated by induration formation (not erythema), is interpreted according to the Table II below (collected from current CDC guidelines).

A TST becomes positive around two months after TB infection. That is why timing of the test is important to get a meaningful result. As written below, a TST done very early in the course of the disease will be false negative. One should wait at least 8 weeks from the last documented exposure before doing a TST.

Table II: Table showing interpretation of TST induration.

Induration (mm)	Significance
0 - 5	Negative
5 - less than 10	This is considered positive in • HIV positive individuals • Recent contact with an open case of TB • Transplant recipient • Patients on other immunosuppressives like TNF-a antagonists • Children with clinical or radiological suspicion of TB In all other persons, this is considered negative. In BCG-vaccinated children, induration of up to 9 mm may be attributed to the vaccine bacilli for the first 10 years of life.
10 - less than 15	This is considered positive in • Persons coming from TB endemic regions like India • Drug abusers • Microbiological lab workers • Health workers • Persons with chronic conditions like diabetes, silicosis, cancer, etc. • Children younger than 5 years • Children older than 5 years if there is increased exposure.
≥ 15	This is considered positive in any person

Sensitivity of TST in humans is estimated to be around 75%. However, in animals, like cattle, the figure is more than 95%.

False positive

False positive results can occur due to:

- ✓ There is infection with other bacteria of the *M. tuberculosis* complex. This includes *M. africanum* or *M. microtii*.
- ✓ There is previous BCG vaccination.
- ✓ Non-tuberculous mycobacteria infection.
- ✓ Incorrect test reporting and interpretation.

Role of prior BCG vaccination in Mantoux test

Previous BCG vaccination may cause false positive Mantoux test although the reaction is usually weakly positive. BCG is an attenuated strain of *M. bovis* and thus also reacts with the *M. tuberculosis* antigens[13]. This vaccine is included in the universal immunization schedule of high-prevalence countries like India. But the USA and most other Western countries do not make it mandatory, and this vaccine is indicated only for certain groups of people in those places.

Status of prior BCG vaccination must be considered while interpreting tuberculin test result. However, the effect of BCG on tuberculin test wanes after 15 or more years[14]. So, if BCG is given at birth, a tuberculin test of the person in adulthood would probably not be a problem. According to a meta-analysis, the relative risk (RR) of a positive tuberculin skin test (TST) is around 3.5 if the test is performed within 15 years of BCG vaccination; but the RR falls to just 1.4 after 15 years[14]. The TST after BCG is more likely to be positive with PPD-RT23 than with PPD-S[14]. Moreover, a strong reaction with induration > 15 mm even in a BCG vaccinated individual is considered significant[14]. The TST positivity after BCG vaccination usually develops 4 - 8 weeks after the vaccine. In some countries, the TST is used to assess the success of the BCG vaccination. If the TST is negative after BCG vaccine, the vaccine is repeated. But India does not have any such follow-up schedule.

For prior vaccinated individuals, the TB blood test (IGRA) is better as it is not influenced by the BCG[15]. As everyone in India is vaccinated with BCG at birth, the IGRA would be a better test of latent TB in this country (see later)[15].

Mantoux test in the paediatric population

In the paediatric population, the recommended dose of PPD is 1 TU of PPD-RT23[16]. The rest of the procedures are same as in adults. Interpretation is as per Table I.

Repeat test

Usually, unless the test has been conducted non-professionally, there is no indication of a repeat test. If needed, repeat test must be done within 1 week of the first test[15]. This is because, the antigen given during the first test may "boost" the reaction of the repeat test. This may give rise to a false positive result in the repeat test.

This "boosting" effect develops within 2 - 3 weeks and lasts up to 18 months, according to an Indian study[16]. So, the repeat test has to be done either very quickly (within 1 - 2 weeks) or after 18 months. Before interpreting any TST, the history of previous testing must be recorded to avoid false positive reporting.

Two step TST

According to the CDC, Atlanta, there is one situation where "two-step" Mantoux testing is done. This is the baseline test of health workers who will undergo future periodic testing. In short, the protocol for two-step test is as follows:

1. The first TST is done as per protocol. If this comes positive, then the worker is infected already at baseline.
2. Then, no more TST is needed and the worker may be further evaluated for infection.

3. If the first TST is negative, then after 2 - 3 weeks, a second TST is done.
4. If the second is also negative, then there is no baseline TB infection.
5. If this second test comes positive, this may indicate a "boosted" response. This result is documented. If future periodic TST is done, those results will be compared to this "boosted" reaction to look for true Mantoux conversion.

False negative

Some people, even after harbouring the TB bacilli, may show absence of reaction to the TST. Some of the reasons are[3]:

1. Infants.
2. Very recent TB infection (less than 2 months).
3. Severe active TB with high bacterial load.
4. Recent live virus vaccination (within 1 month) (so, if a TST is needed, it should either be given on the same day as the live vaccine or we should wait for 1 month).
5. Incorrect method of the TST.
6. Anergy.
7. Very old TB infection (this negative result may become positive after repeat testing due to "boosting": see previous section).
8. Infection with measles, varicella, etc.
9. In very old age, the reaction may not be fully developed after 72 hours. So, in those cases, the reading should be done after 1 more day to avoid false negative results.
10. Malnutrition.

Reversal of TST

This is defined as conversion of a positive reaction to a negative one during a person's lifetime. This is a very rare phenomenon and is said to occur in old age[17]. This can also occur if the initial induration was borderline positive.

Mantoux conversion

This is defined as changing of a previously negative Mantoux to a positive result[18]. Or, an increase in induration size by ≥ 10 mm compared to a previous test. This usually indicates new infection with the TB bacillus.

When should a Mantoux test be avoided?

Like any other biomedical test, a tuberculin test is also subject to confusion if done indiscriminately. There are some situations where the test becomes superfluous. They include[3]:

- Previous confirmed TB (The test can remain positive for a long time after treatment[19]. The exact duration of immunoreactivity is not known with certainty and thus, Mantoux test after a previously diagnosed episode of TB has doubtful value. The test indicates exposure to the bacillus but does not indicate the outcome: bacterial clearance vs. persistence[20]. This is even true for immunosuppressed individuals. So, the Mantoux test cannot differentiate a previously treated case of TB from an untreated one).
- Infants less than 3 months.
- If the first reaction is > 15 mm.
- Clearly apparent active TB disease.
- Previous severe allergic reaction to the solution.
- **Pregnancy:** Here, TST is not absolutely contra-indicated. But it should be done only if the benefit-risk ratio is favourable.

Mantoux test in pulmonary vs extra-pulmonary TB

Generally speaking, the TST should be positive if there is TB antigen exposure at any anatomical area. But it is found that the Mantoux test is more reliable for pulmonary TB. In many extra-pulmonary cases, the sensitivity of the test drops. For example, using the 10 mm cut-off, the sensitivity of TST for TB meningitis is around 50% in one study[21]. For tubercular pleural effusion, the sensitivity is around 65%[22]. The sensitivity of Mantoux test for extra-pulmonary TB has been reported to be less than 50% in a study involving South Asian subjects[23].

Comparison with IGRA

Another test which is now widely available, is the Interferon Gamma Release Assay (IGRA). The basic principle of this test is exposing peripheral blood lymphocytes to synthetic *M. tuberculosis* antigens and assessing the release of interferon-gamma[24]. The T-lymphocytes of a person, already sensitised to TB antigens, will quickly release large quantities of IFN-γ when exposed to antigens like ESAT-6 and CFP-10. The quantity of this interferon in the sample will tell us about the infection. This test has a lot of advantages over TST, like:

1. Requirement of a single blood sample and no need for the patient to return for reading.

2. Higher sensitivity and specificity.
3. Lack of confounding by previous BCG vaccine.
4. Lack of cross reaction with environmental mycobacteria.
5. Quicker (< 24 hours) reporting.
6. *In-vitro* test.

In various studies, both varieties of the IGRA, i.e., the Quantiferon and TB-spot, have consistently shown better sensitivity compared to TST by more than 10 percentage points[25]. So, this test is likely to supersede the Mantoux test in the future. This may also be used to test for vaccine efficiency in public health surveys[24]. But, in a country like India, where resources are limited, wider uptake of the IGRA is still debatable. So, TST will remain an important tool in clinical and public health setting in this country for the near future.

Conclusion

The Mantoux test is an age-old test with proven merits and demerits. It has its limitations; but in a country like India, the test is still useful from both clinical and public health points of view. A clinician should be aware of the nuances in interpretation of the test. The overall diagnosis of tuberculosis should not be based on the TST alone but this test has important complementary role, along with other microbiological tests, in the overall management of the TB epidemic.

Recommendations

- Tuberculin test is still an essential component of tuberculosis management in India but it should not be used alone.
- There are clear age- and co-morbidity specific guidelines for interpretation of the test. False positives and negatives are possible.
- Proper technique is essential for performing the test. Skill development is necessary.
- Where possible, the IGRA test is better, especially in BCG vaccinated persons.

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REVIEW ARTICLE

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Vaccine-induced thrombotic thrombocytopenia (VITT) in COVID-19 vaccination: Demystified

Cynthia Amrutha Sukumar, Ajit Singh**, Nabeela Fatima***, Shreya Jayaram****, Sudha Vidyasagar******

Abstract

The beacon of hope in the face of the COVID -19 pandemic has been the development of vaccinations. However, once these vaccines were administered to the masses, there were some rare complications reported. The most concerning among them was the development of a condition which presented with thrombosis, commonly in the cerebral and splanchnic veins in the presence of thrombocytopenia. This development led to a ban on some specific COVID-19 vaccines in certain countries. This thrombotic thrombocytopenia was elusive due to its clinical presentation that mimicked other diseases including COVID-19 infection.

This review was done following a methodical search in electronic databases including Google Scholar, Springer publication, World Health Organisation guidelines, PubMed and Cochrane. The data has been extracted and presented as a narrative review to help clinicians better understand and identify this post-vaccination phenomenon.

This study revealed a two-fold benefit, diagnostic and therapeutic. While evaluating a patient with thrombocytopenia and thrombosis it is prudent to consider VITT as a differential as it helps avoid a battery of investigations to prove the disease aetiology. Also the duration of anticoagulation can be restricted to 3 months in VITT which otherwise would need to be continued life-long in unexplained thrombotic events.

Key words: COVID-19, vaccination, thrombotic thrombocytopenia, vaccine-induced complications.

Introduction

COVID-19 has had an impact on the life of every individual as well as the global economy. This pandemic has caused unimaginable rates of mortality and morbidity.

From the outbreak of the COVID-19 pandemic in China in 2019, there are 235,242,311 confirmed cases with an active count of 18,418,977. The recovery count was 212,014,726 cases and death of 4,808,608 cases, as of October 2021[1].

Vaccination is a known prophylactic measure to prevent any infectious disease. Throughout history, vaccination has been used as a tool to combat the scourge of viral diseases and epidemics. Since the first appearance of COVID-19 in December 2019, there has been a world-wide race to come up with vaccines for COVID-19. We now have several approved vaccines which include mRNA-based vaccines (Pfizer-BioNTech and Moderna) and recombinant adenovirus-associated vector vaccines (AstraZeneca and Johnson and Johnson) and Whole-Virion Inactivated Vero Cell derived platform technology based indigenous vaccine from Bharat Biotech, India[2].

The mass vaccination campaigns in India and across the globe to ensure vaccination against COVID-19 is a huge effort. Billions of doses are being administered in a short duration to target a reduction in the number of severe cases and deaths. However, there has been a lot of concern regarding the thrombotic side effects of these vaccines. More particularly the AstraZeneca (AZ) vaccine, which though still approved by Medicines and Healthcare products Regulatory Agency (MHRA) and European Medical Agencies (EMA) has been in the eye of controversy due to the thrombotic complications. The first case of thrombosis was identified after the AstraZeneca vaccine in Feb'2021[3].

Justification for Review

With the emergence of this prothrombotic syndrome, several questions have been raised regarding the safety of these vaccines. Though the vaccines hold such promise, the threat of these thrombotic complications seem to overshadow it use among the masses. Therefore, it is imperative to examine the evidence available regarding these vaccine-associated complications and justify the use of these vaccines despite the risk.

Assistant Professor, **Research Associate III for ICMR, ****Intern, *****Professor and Unit Head, Department of Medicine, Kasturba Medical College, Manipal Academy of Higher Education, Manipal- 576104, Karnataka; ***Intern, St. Paul's College of Pharmacy, Turkayajmal - 501 510, Telangana.

Corresponding Author: Dr Cynthia Amrutha , Associate Professor, Department of Medicine, Kasturba Medical College, Tiger Circle Road, Madhav Nagar, Manipal Academy of Higher Education. Manipal- 576104, Karnataka. Tel: 7259415415, E-mail: cynthiaamrutha@gmail.com.

Methodology

First, the authors decided to go for a systematic review in this area, but due to the lack of authentic literature decided to represent the data in narrative review format.

Definition

Vaccine-induced thrombotic thrombocytopenia (VITT): Scully and his colleagues defined VITT as patients presenting with acute thrombosis and thrombocytopenia with elevated D-dimer following a COVID-19 vaccination[7].

Search methods

The data were searched through the popular electronic databases including Google Scholar, Springer publication, World Health Organisation guidelines, PubMed and Cochrane. All the articles were arranged as per the information they have provided and arranged in a systematic way.

Population

Data were searched for the population of > 18 years of age who received any COVID-19 vaccination irrespective of mortality status. The data for the population < 18 years of age and who did not receive any COVID-19 vaccination were excluded.

Outcomes observed

Occurrence of thrombotic events following COVID-19 vaccination

Data extraction keywords

VIIT, Vaccine-induced thrombotic thrombocytopenia (VITT), thrombotic events and COVID vaccination, MACE following COVID vaccination, adverse events of COVID vaccinations.

Epidemiology of VITT

The incidence of VITT after 1st dose is 1.3 per million doses; and after the second dose is 2.7 per million doses with the age group of 18 - 49 years[4].

After the first case report in February 2021, in early April 2021, 6 cases of Cerebral Venous Thrombosis (CVT) were reported. The age group was between 18 and 48 years and the onset of symptoms was 6 - 13 days post-vaccination. FDA and CDC suspended the use of the Johnson and Johnson vaccine. By the end of April 2021, 169 cases of CVT and 53 cases of splanchnic vein thrombosis (SVT) were reported[5].

Due to the reports of VITT, Germany, Spain, Italy, the UK, France restricted the use of Vaxzervria (CoviShield in India). The UK advisory board suggested Vaxzervria should not be given to the age group below 30 years and whoever has taken the first dose may take a different vaccine for 2nd dose. In March 2021, Australia also suspended the use of Vaxzervria[5]. It is interesting to note that BBBP-CorV (SinoPharm, Shanghai, China), CoronaVac (Sinovac Biotech, Beijing, China), BBIBP-CorV and WIBP-CorV (Sinopharm, Beijing, China) did not report any cases of VITT.

In May 2021, the product information of Vaxzevria was updated with regard to the very rare risk of thrombosis (formation of blood clots in the blood vessels) with thrombocytopenia (low blood platelets) syndrome (TTS).

However, by August 2021, the European Medicines Agency (EMA) thoroughly assessed the data on the quality, safety, and efficacy of the vaccine and recommended granting a conditional marketing authorisation for people aged 18 and above.

Germany faced a different challenge as they attempted to use the surplus vaccination (over 2 milllion) on a reluctant population. This led the mass donation of unused vaccines by Germany to low-income countries in an effort to utilize vaccines before their expiry.

As of late September 2021, The Italian authorities also announced that all versions of the AstraZeneca vaccine are considered equivalent to the other vaccines that have already been approved for use by the country's authorities. The incidence of VITT among the various COVID-19 vaccines (AstraZeneca, Pfizer, Moderna, Sputnik, Covishield and Covaxin is shown in Table I.

Table I: Reports of thrombotic events per dose administered (data up to 31 July 2021, adapted from authentic resources).

Vaccine name	Total dose administered (Approx.)	Thromboembolic events report
VaxZevria (AstraZenca SKBio)	34 million	222
Pfizer BioNTech (USA)	54 million	35
Moderna (USA)	4 million	5
Janseen Ad26.CoV2 (Johnson and Johnson, USA)	7 million	6
Gamaleya Sputnik V (Russia)	943,000	2
Covishield (India)	68 million	26
Covaxin (India)	7 million	0

Pathogenesis

The indication of the similarities in Heparin-induced

thrombocytopenia (HIT) and Vaccine-Induced Thrombotic Thrombocytopenia (VITT) is due to the temporal association of symptom onset after vaccination, thrombosis at arterial and venous sites, as well as the thrombocytopenia[4].

Poi-Wei Chen *et al* proposed a mechanism that interaction between PF-4 and components of the COVID-19 vaccine could result in immune complexes[5].

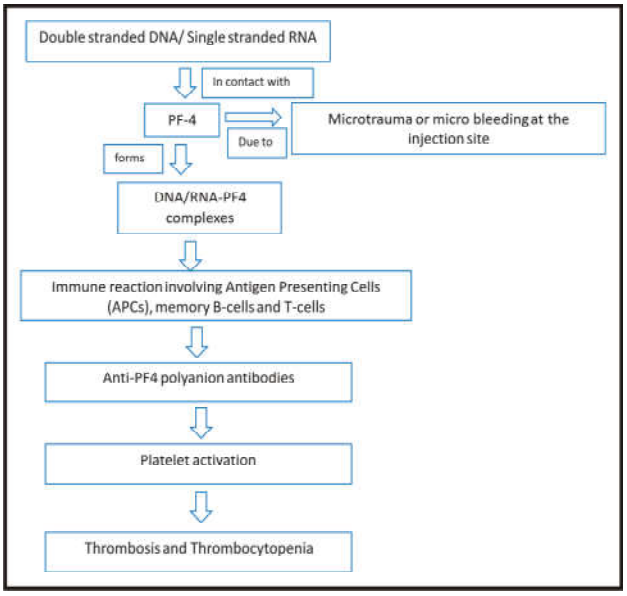


Fig. 1: *Proposed pathophysiology of VITT.*

A hypothesis stated that an autoimmune condition or pre-existing hypercoagulable status of an individual may trigger resulting in a cascade of thrombocytopenia and thrombosis. However, this was challenged when in a study on 11 patients, only 1 patient was found to have pre-existed Von Willebrand disease, anticardiolipin antibodies, and factor V Leiden[5]. Scully and his colleagues evaluated 23 patients with VITT and noted that they had no pre-existing prothrombotic condition or events to corroborate this hypothesis[7].

Schultz *et al* included 5 healthcare workers who presented with vaccine-induced thrombosis (four cases of CVT and one of portal vein) after 7 - 10 days following vaccination with first dose of AstraZeneca vaccine. All these patients had high levels of PF4-polyanion complexes[8].

Gonsalge *et al* reported an association of local tissue micro-trauma that occurred post-vaccination. They presented a hypothesis that contact of adenoviral DNA and PF-4 could lead to immuno-thrombosis due to increased anti-PF4 autoantibody. They proposed the concept of the Spike variant in the adenoviral genetic material that binds to endothelial cells and triggers platelet activation leading to thrombosis (as shown in Fig. 1)[9]

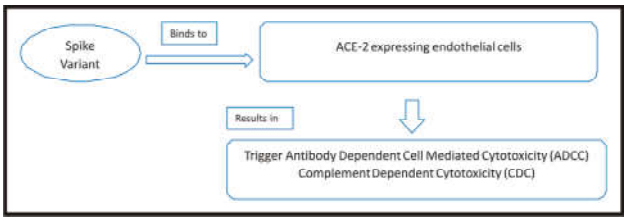


Fig. 2: *Pathophysiology of VITT involving the spike protein.*

Another possible hypothesis is that PF-4, a chemokine stored in platelet alpha granules is released due to activation of platelets and bind to high-affinity polyanions. This hypothesis was based on HIT, where heparin acts as hapten, i.e., production of specific antibodies is spurred due to the heparin binding to PF-4[10].

Predisposing factors

Host factors: This includes the female sex as reflected from early case reports this year. One series of VITT cases showed a female preponderance for 9 of 11 patients and another series reported 4 of 5 cases to be female[8,13]. Younger age also seems to have a predilection for VITT. It is suspected that as most of the vaccinated individuals were young women in the early phase of the vaccination campaigns, it is likely that the increased number of cases of VITT are due to a biased representation[8].

Vaccine factors: Two adenoviral vector-based vaccines have been implicated in causing VITT. The ChAdOx1 nCoV-19 (AstraZeneca, University of Oxford, and Serum Institute of India) has been reported to have more association with VITT[8]. The Ad26.COV2.S (Janssen; Johnson and Johnson) vaccine also has been implicated but to a lesser extent. The highest incidence of VITT in AstraZeneca as 1 in 26,000 in the Norwegian population. The Centre for Disease Control (CDC) reported that the Jannsen vaccine showed the incidence of associated VITT to be a mere 1 in 53,3333 vaccinated individuals.

Clinical features

Following the definition for VITT proposed by Scully and his colleagues, the largest study on cerebral venous thrombosis following vaccine for COVID-19 was done in the UK and a modified definition for VITT was proposed. They suggested that cases of CVT can be considered VITT-associated if the lowest platelet count recorded during admission was below 150×10^9 per L and, if the D-dimer was measured, the highest value recorded was greater than 2000 ¼g/L[11].

The clinical manifestation of VITT is essentially the presence of the triad as shown in Fig. 3 occurring from 5 to 30 days

following COVID-19 vaccination.

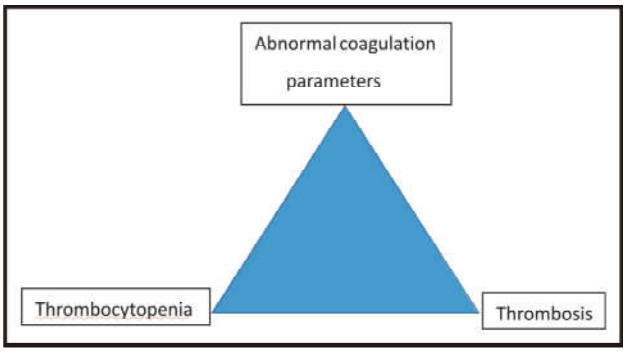


Fig. 3: *Graphical representation of VITT.*

In a large prospective cohort study done in UK, it was found that the mean baseline platelet count among the patients with VITT was only 47,000/cumm. Even though 5% of these patients had normal platelet count at the time of presentation, their counts dipped below normal during the course of hospitalisation. Thrombosis most commonly manifested as thrombosis of the cerebral veins (in upto 50%) of the patients. Deep veins of the legs were the next most common site of occurrence of thrombosis in these patients. Splanchnic veins are the most common site of thrombosis in the portal circulation[7]. The most common site for arterial thrombosis is the middle cerebral artery territory and less commonly the coronary arteries[14].

It was also noted that thrombosis with low platelet counts led to secondary complication of intracranial haemorrhage more commonly than thrombosis with normal platelet counts. Multivariate analysis identified the baseline platelet count and the presence of intracranial haemorrhage as being independently associated with death[7].

A study by Greinacher *et al* included 9 patients between 22 - 49 years. They had developed symptoms 4 - 16 days post-vaccination. Of these patients, 7 had CVT, 1 had pulmonary embolism, 1 had concurrent splanchnic and cerebral vein thrombosis. 4 patients succumbed to their complications and their reports showed positive anti-PF-4 antibodies and positive platelet activation assay[14].

Coagulation abnormalities such as DIC also can manifest in VITT. The spectrum of presentation ranges from clinically severe intracranial haemorrhage to mild bleeding tendencies such as petechiae and purpura. A majority of patients only have a transient thrombocytopenia.

As VITT clinically mimics a host of other conditions including Heparin-induced thrombocytopenia and Thrombotic thrombocytopenia purpura, it was important to have criteria to define this condition. The Case Definition Criteria for Vaccine-Induced Immune Thrombocytopenia and Thrombosis (VITT), according to an Expert Haematology Panel has been described in Table II[7].

Table II: Criteria for diagnosis of VITT.

Type of VIIT Description	Criteria
Definite VIIT	All five of the following criteria: 1. Onset of symptoms 5 - 30 days after vaccination against SARS-CoV-2 (or ≤ 42 days in patients with isolated deep-vein thrombosis or pulmonary embolism) 2. Presence of thrombosis 3. Thrombocytopenia (platelet count < 150,000 per cubic millimeter) 4. D-dimer level > 4,000 FEU 5. Positive anti-PF4 antibodies on ELISA
Probable VIIT	D-dimer level > 4,000 FEU but one criterion not met (timing, thrombosis, thrombocytopenia, or anti-PF4 antibodies) or D-dimer level unknown or 2,000 - 4,000 FEU and all other criteria met.

Once the diagnosis of VITT has been confirmed, the clinical severity can be assessed by the 4Ts score which is adapted from the scoring system for Heparin-induced thrombocytopenia. The scoring chart is summarised in Table III. This table is adapted from the 4Ts score for HIT, which has been validated in various populations. This adapted scoring system has not been validated, and the incidence of VITT is very low, making validation challenging. As a result, the interpretations do not carry a specific risk for VITT or correlate with a specific likelihood of positive PF4 antibody testing.

Table III: The 4TS score for clinical severity in VITT.

Variable	Score
Thrombocytopenia	
Platelet count fall > 50%	2
Platelet count fall 30 - 50%	1
Platelet count fall < 30%	0
Timing of onset	
Within 4 - 16 days of vaccination	2
> 2 weeks of vaccination	1
No history of vaccination	0
Thrombosis	
New thrombosis post-vaccination	2
Progressive/recurrent thrombosis	1
No thrombosis	0
Other causes of thrombocytopenia	
None	2
Possible	1
Definite	0
Total score	
0 - 3: Low probability	
4 - 5: Intermediate probability	
6 - 8: High probability	

Table IV: Comprehensive clinical overview of VITT after COVID-19 vaccination[15-17].

Age/Gender	Vaccine	Symptoms	Laboratory tests	Treatment
44 Male[14]	AstraZeneca	Day-8: Fevers, fatigue, foggy head, abdominal discomfort, increased bowel frequency.	Low platelet count; elevated D-dimer; CT-thrombosis with complete occlusion of portal and splenic veins and protrusion of thrombus into superior mesentric vein.	Fondaparinux,IVIGMethyl-prednisolone
26 Female	AstraZeneca	Day-8: Severe headache, thrombocytopenia.	Slightly elevated D-dimer, positive PF-4 antibodies.	Platelet transfusion, Heparin, IVIG Dexamethasone Apixaban
29 Female[15]	Astrazeneca	Severe headache, left orbital swelling, blurred left eye vision, fever.	Thrombocytopenia, high D-dimer, elevated CRP, MRI: hype T2 signal in left superior ophthalmic vein (SOV); PF-4: positive	IVIG Antibiotics Rivaroxaban Prednisolone
30 Female[13]	Janssen	Day-10: Headache, neck pain Day-17: lower extremity pain and weakness	Thrombocytopenia, CT: subtle increasing density of right transverse and sigmoid sinuses suspective of dural sinus thrombosis; Duplex U/S: Acute DVT involving posterior tibialis and popliteal veins; MR and CT Venography: large near occlusive thrombus in right transverse sinus extending to right sigmoid sinus & jugular bulb.	Agartoban Bivalirudin Apixaban
62 Female[13]	Pfizer	Day-9: headache and vomiting	CT: increasing size of haemorrhagic right cerebral venous infarcts with early hydrocephalus requiring decompressive craniotomy.	Unfractionated Heparin Low Molecular Weight Heparin Warfarin

There was also an association noted between the presence of the above mentioned predictors of VITT and mortality. In the UK prospective cohort study, it was noted that intracranial haemorrhage and cerebral venous thrombosis had a univariate Odd's ratio of 4.7 and 2.7 respectively with a 95% CU. Thrombocytopenia (every 50% drop) and low fibrinogen level had a univariate ODD's ratio of 1.7 each[7].

Apart from the AstraZeneca vaccine, some mRNA vaccines-Moderna and Pfizer BioNTech also were associated with immune thrombocytopenia and bleeding with thrombosis as reported in August 2021[14].

A comprehensive summary of the reported cases has been presented in Table IV.

Autopsy studies

Several autopsy studies have also been done on patients with post-vaccination symptoms and venous thromboembolism at unusual sites, i.e., cerebral and abdominal veins. There was a concomitant presence of haemorrhage and consumption coagulopathy with low plasma fibrinogen, highly elevated D-dimer, and strongly positive PF-4/heparin antibodies. All of this was associated with poor prognosis and higher mortality.

Anatomic dissection showed a catastrophic picture with multiple sites of venous thrombosis with intracranial bleeding. The usual sites showed involvement of large venous vessels far more extensive than predicted imaging. Microvascular findings showed vascular thrombotic occlusion in multiple organ microcirculation and increased inflammatory infiltrates.

Immunohistochemical analysis was done and vascular and perivascular expression of adhesion molecules such as VICAM1 was noted. Also, the presence of $CD66b^+$, $CD163^+$, and $CD61^+$ activated inflammatory cells indicated the activation of the innate immune system and complement pathway. This promotes inflammatory processes leading to microvascular damage of multiple organs[18-20].

The clinical history and course in the hospital for 2 patients of VITT who succumbed are summarised in Fig. 4a and 4b.

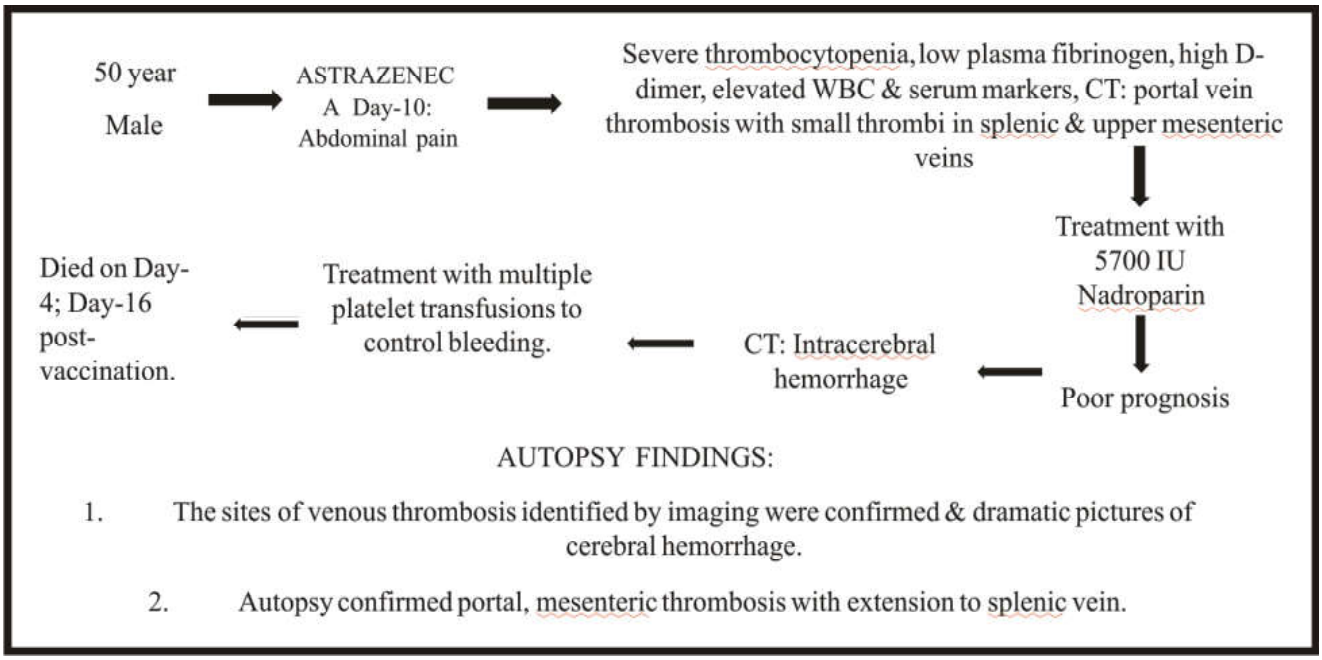
Evaluation of VITT

VITT must be suspected in all patients who have received vaccine with an interval of 5 to 30 days associated with thrombosis and thrombocytopenia.

However, the diagnosis needs laboratory evaluation which includes:-

1. Complete blood count: To look for thrombocytopenia (to look for fall in serial counts).
2. Coagulation parameters: Prothrombin time (PT) and activated partial thromboplastin time (aPTT), D-dimer and Fibrinogen.
3. PF4 antibody testing: Confirms the diagnosis of VITT in conjunction with the thrombocytopenia and D-dimer.

   However, it must be remembered that PF4 antibody can be positive with normal platelet counts and the patients are usually asymptomatic in this cohort. PF4 assays can be done using the Enzyme-linked immunosorbent assay method, Seratonin release assays and rapid HIT assays[7].



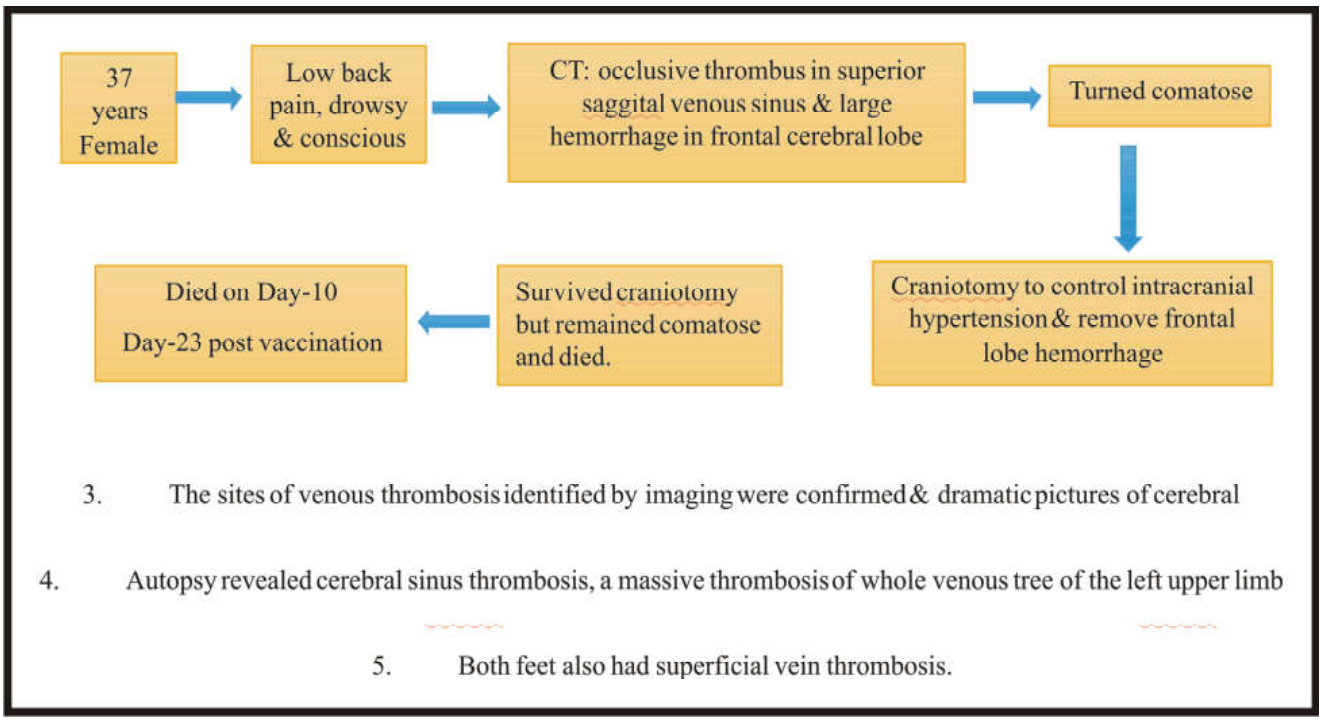


Fig. 4a and 4b: *Summary of autopsy findings in a case of VITT.*

A diagnosis of VITT is made with a positive PF4 ELISA in the clinical background of post-COVID-19 vaccine thrombosis and/or thrombocytopenia.

Imaging to confirm sites of thrombosis in the brain, thorax and abdomen may be done according to the clinical presentation of the patient.

Differential diagnosis

The other causes that have a similar presentation to VITT should be considered in patients presenting with thrombosis and thrombocytopenia following vaccination especially if the PF4 antibody is negative. The following conditions should be considered.

1. Heparin-induced thrombocytopenia: Classically presents following exposure to heparin and the thrombocytopenia resolves following cessation of heparin.
2. Immune thrombocytopenia: Not associated with thrombosis. Coagulation profile remains unaltered.
3. Thrombotic thrombocytopenic purpura: Presents with thrombocytopenia and microvascular thrombosis with

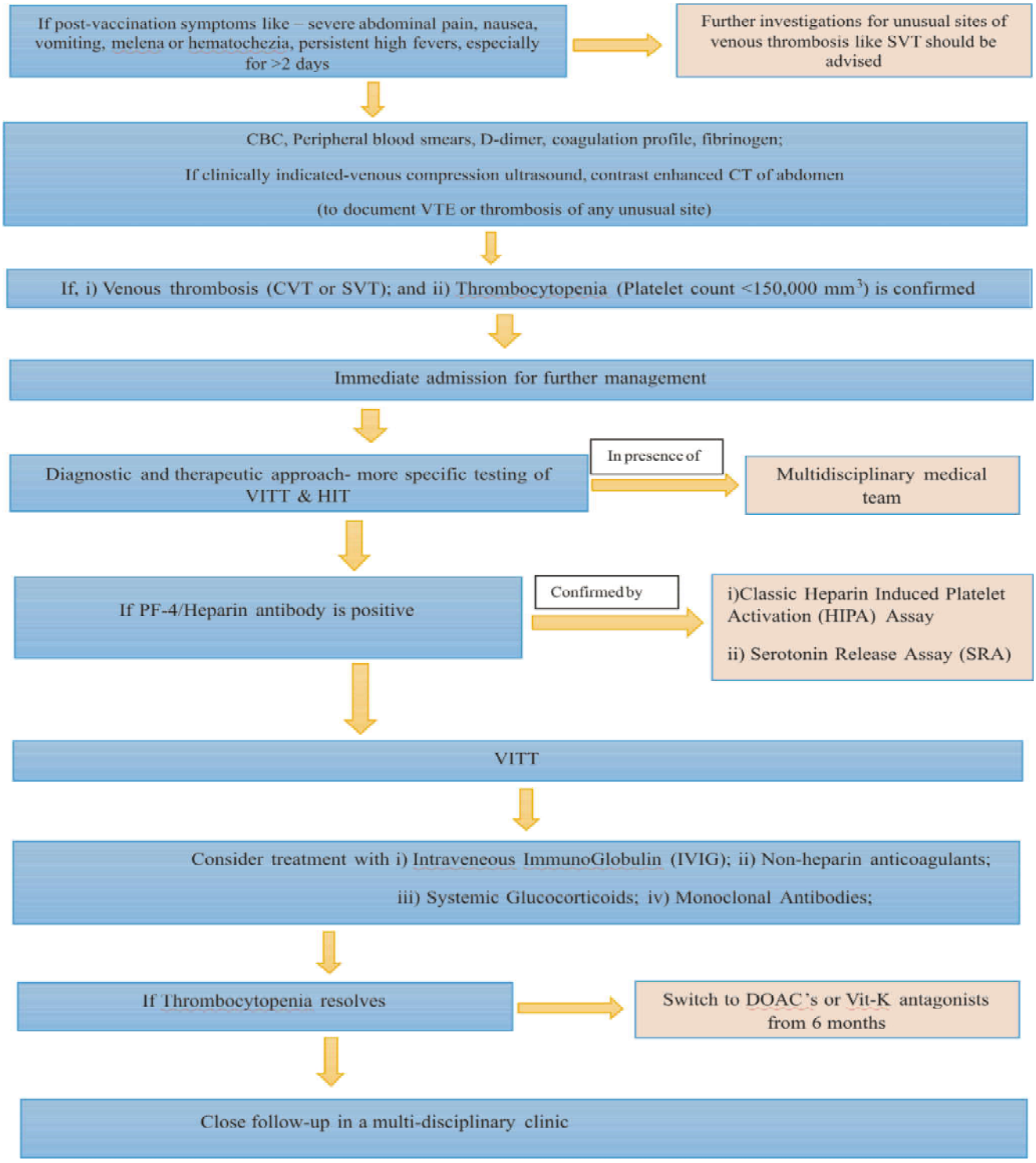


Fig. 5: *Overview of management in VITT.*

neurologic/kidney/cardiac involvement. Coagulation profile remain normal.

4. COVID-19 infection: This must be ruled-out in all cases of VITT as the COVID-19 infection itself can cause thrombosis and thrombocytopenia. VITT causes thrombosis in cerebral and splanchnic circulation commonly while COVID-19 involves the pulmonary veins and the deep veins of the lower limb.
5. Other causes of thrombocytopenia like sepsis, splenomegaly and inherited disorders.
6. Other causes of thrombosis like oral contraceptive pills, cancer, pregnancy, surgery and trauma.

Treatment

There is no specific therapy that is efficacious for the management of VITT. For effective therapy, it is crucial to assess the phenotype, risk factors, natural history, early detection, and management of VITT[10]. Clinicians should be familiar and vigilant in creating awareness among colleagues regarding the triggers of VITT along with the clinical signs and laboratory investigations[18]. Treatment options may include Monoclonal antibodies (Rituximab, Eculizumab); Direct thrombin inhibitors (Bivalirudin, Argatroban, Dabigatran); Direct Factor Xa inhibitors (Rivaroxaban, Apixaban, Edoxaban); Indirect Factor Xa inhibitors (Fondaparinux); Steroids (Prednisolone, Methyl-prednisolone, Dexamethasone)[13].

A life-saving approach could be early administration of high dose Intravenous immunoglobulin in addition to non-heparin anticoagulation which may help in the interruption of thrombosis cascade. All patients of VITT should be offered high-dose IVIG unless contraindicated. The recommended dose is 1gm/kg intravenously once a day for 2 days. It acts by promoting autoantibody binding to cellular receptors and thereby blocking platelet activation. It is crucial to monitor the platelet count during hospitalisation and after discharge as thrombocytopenia can occur even after the course of IVIG is completed.

In a Canadian study, three patients with VITT were administered high dose IVIG and dramatic improvement in thrombocytopenia was noted[21]. Another study on VITT patients with thrombotic manifestations also concurred with the Canadian study.

IVIG not only halted the platelet activation but also prevents occurrence of new thrombosis or worsening of progressive thrombus[22].

Anticoagulation is the mainstay of treatment in VITT. Even cerebral venous thrombosis (CVT) associated with haemorrhage is not a contraindication to anticoagulation as this finding is attributable to increased venous "back pressure" and often resolves rapidly with anticoagulation. Anticoagulation is also indicated in patients with VITT without thrombosis and patients with strong clinical suspicion of VITT who are awaiting laboratory confirmation[20.] Anticoagulation is a challenging task as most of the reported deaths among the patients with VITT were due to intracerebral haemorrhage. Fig. 5 presents an overview to the approach and management of VITT.

Steroids are used as second-line drugs in the management of VITT. Steroids are thought to counter the synthesis of new antibodies thereby interrupting the platelet activation and the resulting thrombosis. Despite this hypothesis, there are no large studies to corroborate the benefits of steroids in VITT. There is only some limited data available in this regard from its use in specific cases only. There is more benefit of steroid when use concurrently with IVIG. In the study by Schulz *et al*, 80% (4 out of 5) were treated with IVIG and steroids. Among the treated patients, half of them succumbed to VITT despite treatment. In another study by George *et al*, there was a better response to treatment with IVIG and steroids reported. As these studies have reported the use corticosteroids with IVIG, the stand alone benefit of steroids in VITT is still uncertain.

Platelet transfusions should be minimised (except in cases of critical bleeding tendencies) in VITT as it can trigger further platelet activation[1]. There is no role for aspirin in the treatment of VITT as it has no function in the prevention of platelet activation due to PF4 antibody.

The natural history of the antibody is not known. Hence, such cases require close follow-up with clinical and laboratory parameters for risk of recurrent thrombocytopenia.

Therapeutic plasma exchange (TPE) and immunosuppression can be considered for refractory VITT or with associated complications such as cerebral vein thrombosis (CVT) or multiple thromboses with evidence of excessive platelet activation (platelet count <30,000/microL)[23].

Prevention

Though the pathophysiology of VITT leads to thrombosis, there have not been any reports of VITT in individuals with HIT or thrombosis caused by another risk factor. Also, the mechanism of VITT differs from HIT (due to different PF4 epitope) and other types of thrombosis (which have different mechanisms). Hence there is no recommendation at present for patients with history of thrombosis to avoid adenoviral vaccines.

Individuals who have received the first dose of adenoviral vaccine without any VITT related complications can take the second booster without hesitation. According to a review based on data from the AstraZeneca database from Europe and UK, it was noted that there were 399 cases of VITT following the first dose but only 13 after the second-dose. This shows the sharp decline in the incidence of cases following the second-dose. Thus, it is advisable for individuals to take the second-dose as they can avail the full benefit of the superior efficacy of this vaccine on completion of the booster dose[24].

Keeping this in mind, UK has maintained that the second vaccination must be given to all patients who have received the first-dose of the AstraZeneca vaccine without any complications. However, in Germany, France and Canada the authorities have restricted the use of the AstraZeneca vaccine to patients older than 60 years, 55 years and 40 years respectively.

VITT and booster dose

In the event of VITT, a booster or annual vaccination (usually advised to protect from COVID-19 and emerging variants) comes into question. Should patients who have had VITT after the first- or second-dose of COVID-19 consider taking a booster dose?

The Centre for Disease Control (CDC) recommends repeat immunisation with the same vaccine in the absence of any VITT event with the first vaccine. However, those who developed VITT from an adeno-virus mediated vaccine can be switched to an mRNA-based vaccine for the second dose. There has also been a recommendation to consider non adeno-virus mediated vaccines as boosters.

Despite sparse evidence, several countries have recommended a "heterologous prime boost regimen" where the second-dose or booster dose is replaced by an mRNA based vaccine when the first vaccination has been done using the AstraZeneca vaccine.

Presently, there is no data available on booster dose for COVID-19 in patients with VITT.

Implications and Conclusion

Although we do see cases of venous and arterial thrombosis in clinical practice, we need to be aware of its presentation following vaccination. We should be aware about the recent literature and guidelines about the diagnosis and management of VITT as it will help us narrow down the differential diagnosis for arterial and venous thrombosis and thereby aid in tackling this emerging problem more efficiently. Hence, details of vaccinations and predisposing risk factors such as hormone therapy/oral contraceptive use must be actively sought for such cases as they will guide the clinician to make a correct and timely diagnosis. Prodoromal symptoms like lethargy, prolonged fever and headache must not be neglected following vaccination.

Treatment in VITT is wrought with hurdles such as bleeding which is challenging due to the competing goals of halting the bleed and preventing thrombus. The significance of PF4 assays in the management of VITT is also unclear. The cornerstone of treatment remains anticoagulation. The duration of anticoagulation is decided along the same lines as for HITT. VITT with thrombosis will warrant anticoagulation for 3 months (after normalisation of platelet counts) while VITT without thrombosis requires anticoagulation until 4 to 6 weeks after normalisation of platelet counts. Newer oral anticoagulants are preferred during the period of thrombocytopenia over vitamin K antagonists like Warfarin.

This review concluded that VITT has become an important differential diagnosis to consider in the light of the current pandemic, especially to avoid a battery of unnecessary investigations to prove aetiology. Also, diagnosing VITT also restricts the duration of anticoagulation to 3 months which otherwise would have been indefinite (if aetiology remains unproven). With this review we have attempted to demystify the prejudices and myths associated with VITT and to present it as a new and treatable condition in the post-vaccination period.

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CASE REPORT

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Autoimmune Encephalitis: Look Beyond the Obvious

Pooja Gautam, Shah Yash Rakesh**, Sumeet Singla***, S Anuradha*****

Abstract

Anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis is a life-threatening autoimmune condition caused by antibody production against NMDA receptors leading to its dysfunction. The disease generally affects young females and is frequently associated with neoplasms like ovarian teratoma. Early diagnosis is often missed, as patients may present with psychiatric manifestations. We report the rare case of anti NMDAR encephalitis in a 13-year-old female who presented with headache, vomiting, fever, altered sensorium, and involuntary movements. The patient responded well to treatment.

Key words: Autoimmune encephalitis, Anti-NMDAR, paraneoplastic, dyskinesia.

Key message

- *Autoimmune encephalitis is an uncommon condition mostly affecting young girls.*
- *Due to it's protean manifestations, there is diagnostic confusion and delay; patients may be labelled as psychiatric disease, epilepsy, or infectious (tubercular/viral) disorder.*
- *A subacute onset syndrome of psychiatric, cognitive and behavioral abnormalities, exclusion of commoner etiologies and CSF/serum autoantibody testing can reveal the diagnosis.*
- *With correct recognition, the treatment is fairly simple and clinical features are fully reversible with gratifying results.*
- *A search for underlying malignancy must be carried out in every patient of autoimmune encephalitis.*

Introduction

Anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis is a life-threatening autoimmune condition caused by antibody production against NMDA receptors leading to dysregulation of neurotransmission. Described initially by Dalmau *et al*, it is four times commoner in females and 37% of the patients are < 18 years at presentation[1] with an estimated prevalence of 13.7 per 1,00,000 population[2]. Clinical presentations include cognitive or memory deficits, altered consciousness, seizures, movement disorders, and neuropsychiatric symptoms[3,4]. It is highly responsive to treatment if recognised early, nearly 75% of patients recover completely[1]. It can be a para-neoplastic manifestation of a tumour, most commonly, an ovarian teratoma[5-8]. The relapse rate is 15.9% with most (82.0%) patients experiencing it in 24 months[9]. We report Anti NMDAR encephalitis in a 13-year-old girl who presented with encephalitis syndrome and involuntary movements.

Case report

A previously healthy 13-year-old girl, resident of Delhi, presented to the emergency department with complaints of headache for 3 months, vomiting for 10 days, fever for 6 days and altered sensorium for 3 days. There were behavioural disturbances in terms of agitation, decreased verbal output and non responsive to commands for the last 3 days. There was no history of weight loss, cough, or contact with a case of tuberculosis. There were no history of similar complaints or any long-standing psychiatric illness in the past.

On examination, the patient was conscious, but disoriented (agitated). Her vitals were within normal range. She was febrile (axillary temperature 101° F). General physical examination was unremarkable. The girl exhibited involuntary movements such as orofacial dyskinesia and tremors in the right hand. Terminal neck rigidity was present, but Kernig's/Brudzinki's sign were absent. During the hospital stay, she had one episode of generalised tonic clonic seizure. A provisional diagnosis of meningo-encephalitis was made and the patient was started on intravenous ceftriaxone, vancomycin, acyclovir, dexamethasone and mannitol. A lumbar puncture and was performed. The results of CSF analysis, laboratory investigations, radiological investigations and EEG results

Senior Resident, **Post-Graduate, ***Professor, ****Director Professor, Department of Medicine, MAMC and LNJP Hospital, New Delhi - 110 002.

Corresponding Author: Dr Pooja Gautam, Senior Resident, Department of Medicine, MAMC and LNJP Hospital, New Delhi - 110 002. Phone: , E-mail: Pooja.1919149@gmail.com.

are compiled in Table I.

Table I: Laboratory Investigations of the patient:

Parameter	Value
CSF total cell count	50 cells/mm3
CSF differential count	50% polymorphs, 50% lymphocytes
CSF sugar/ protein	54/274 mg/dl
CSF Adenosine Deaminase (ADA)	3.8 IU/L
CSF CBNAAT for *M. tuberculosis*	Negative
CSF Cryptococcal Antigen	Negative
CSF Gram's stain	Negative
CSF Antibody for HSV and JE virus	Negative
HIV Antibody	Negative
Hepatitis B virus	Negative
Hepatitis C virus	Negative
ANA by ELISA	Negative
Mantoux	3mm
FT3/FT4/TSH	Normal
Anti TPO (IU/L)	28.5 (0-20)
Serum Ferritin (ng/mL)	92.7 (17-464)
NCCT head	Normal
MRI Brain with MR spine	Normal
Ultrasound Whole abdomen	Normal
FDG-PET scan	Normal
EEG	Normal

CSF: Cerebrospinal fluid, ADA: Adenosine deaminase, CBNAAT: Cartridge based nucleic acid amplification technique, HSV: Herpes simplex virus, ANA: Anti-nuclear antibody, ELISA: Enzyme linked immunosorbent assay, TPO: Thyro-peroxidase antibodies, NCCT: Non contrast computerised tomography, MRI: Magnetic resonance imaging, FDG-PET: Fluorodeoxyglucose-positron emission tomography, EEG: Electroencephalogram.

The CSF for anti NMDAR antibodies was strongly positive by an indirect immunofluorescence assay. Other antibodies in the CSF like α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 1 ($AMPAR_1$), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 2 ($AMPAR_2$), contactin-associated protein-like (CASPER), leucine-rich glioma-inactivated 1 (LG-1), and gamma-aminobutyric acid B ($GABA\ B_2$) were all negative.

The patient fulfilled the definitive criteria for Anti NMDAR encephalitis[3]. She was started on IV methylprednisolone pulse therapy at a dose of 1 g for three days along with IV immunoglobulin G (IVIG) 400 mg/kg/day for five days. She made a remarkable clinical recovery and the abnormal behaviour and involuntary movements completely subsided by day 8 - 10 of treatment initiation. Since the patient had a severe episode at presentation and as the disease has a high relapse rate, she was given further immunosuppression with Rituximab at a dose of 375 mg/m^2 once in a week IV infusion for four weeks. Screening for an underlying neoplasm via ultrasound and FDG PET scan was done; but no neoplasm was detected. The patient is doing well under follow-up.

Discussion

Anti-NMDAR encephalitis is the second most common cause of autoimmune encephalitis after acute disseminated encephalomyelitis (ADEM)[10]. However, it is not a diagnosis that is entertained frequently by physicians. This is probably due to a lack of awareness and challenges in confirming the diagnosis. It generally presents with a prodrome of fever and headache with seizures, cognitive and memory deficits along with motor disorders[11]. Due to the neuropsychiatric manifestations, patients may present first to the psychiatrists and an early diagnosis may be missed.

Our patient was initially managed as suspected meningoencephalitis (antibiotics, antiviral, steroids and mannitol) which is also common in childhood and adolescence. However, despite empirical treatment she did not improve adequately. Considering the non-resolution of symptoms, investigations for other causes of encephalitis/ encephalopathy were done.

Our patient fits the definitive criteria for the diagnosis of anti-NMDAR encephalitis (mentioned in Table II), presenting with psychiatric symptoms, speech dysfunction and orofacial dyskinesia.

Table II: Diagnostic criteria for anti-NMDA receptor encephalitis.

Probable anti-NMDA receptor encephalitis
All three criteria must be met:
1. Rapid onset (< 3 months) of at least four of the six following major group of symptpms
• Abnormal behaviour or cognitive dysfunction
• Speech dysfunction
• Seizures
• Movement disorders, dyskinesia, or abnormal postures
• Decreased level of consciousness
• Autonomic dysfunction or central hypoventilation
2. Atleast one of the following laboratory results:
• Abnormal EEG
• CSF with pleocytosis or oligoclonal bands
3. Reasonable exclusion of other disorders
Definite anti-NMDA receptor encephalitis
1. IgG anti-GluN1 antibodies in the presence of one or more of the six major group of symptoms, after reasonable exclusion of other disorders

NMDA: N-methyl-D-aspartate, EEG: Electroencephalogram, CSF: Cerebrospinal fluid, IgG: Immunoglobulin G.

As per literature, almost 45% - 53% of the patients with anti-NMDAR encephalitis have a normal MRI[11,12]. The most frequent and most specific EEG finding is a diffuse slowing pattern with no epileptiform discharges and extreme delta brush pattern respectively[13,14]. As antibody testing in serum is less reliable (100% sensitivity for CSF vs 85% for serum), anti-NMDAR IgG testing should always be done with CSF[4]. In our case, CSF anti-NMDAR antibody was strongly positive. Detection of antibody is important especially when MRI and EEG findings are normal.

Since anti-NMDAR encephalitis is frequently associated with paraneoplastic syndromes like ovarian teratoma, imaging should be done to rule this out. The detection of an ovarian teratoma is age dependent; approximately 50% of female patients > 18 years have unilateral or bilateral ovarian teratomas, while 9% percent of girls < 14 years have a teratoma[4].

Our case is noteworthy because of the presentation mimicking an infective meningoencephalitis with orofacial dyskinesia and tremors in a young girl. Ruling-out infective causes, poor response to presumptive treatment, classical neurological manifestations (altered consciousness, seizure, movement disorders) and a normal neuroimaging prompted us to strongly consider the possibility of anti-NMDAR encephalitis.

Conclusion

Anti-NMDAR encephalitis is the 2nd most common cause of autoimmune encephalitis. A young female presenting with neuropsychiatric manifestations, who does not respond well to empirical antibiotic therapy, should prompt a high index of suspicion for this. Investigations such as MRI, EEG, and lumbar puncture for antibody analysis, facilitates early and accurate diagnosis. Timely diagnosis and treatment enable a good clinical outcome.

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CASE REPORT

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Congenital Morgani Hernia Presenting as Shortness of Breath and Chest Pain

Ruchi Arora Sachdeva, Shailly**, Sahil Singh***, Nikhil Kirar***, Manas Kamal Sen*, Kamran Choudhary**, Mona***

Abstract

Background: Morgagni hernia is a very rare type of congenital diaphragmatic hernia. Its reported prevalence in literature is only 2 - 3%. There is a congenital defect in the anterior part of the diaphragm, which results in penetration of abdominal organs into thorax and results in symptoms. Since it is a congenital disease, it can be detected earlier during foetal life by routine ultrasonography or late during adolescent life. Late diagnosis in adults is extremely rare. Definitive treatment is surgical repair, which should be done in all cases – symptomatic or asymptomatic – to avoid life-threatening complications, like volvulus, strangulation, incarceration, or small bowel obstruction. In view of rarity of the condition we are reporting this case which we have seen recently, presented to us with shortness of breath and chest pain.

Key words: Morgagni hernia, diaphragmatic hernia; chest pain, respiratory distress.

Introduction

The Italian anatomist Giovanni Battista first described Morgagni hernia (MH) in 1769 as a diaphragmatic hernia on an anterior side, originating from the costo-sternal trigones, which is a triangular space between the muscles originating from the xiphi-sternum and the costal margin of the diaphragm and protruding into the central tendon[1]. The most common contents of the hernia sac are abdominal visceral organs including the omentum, followed by the colon, stomach, small bowel, and part of the liver[2]. MH can be present on either side of the sternum; but, it is more commonly found on the right side. Most cases are asymptomatic. In symptomatic cases, the most common presenting symptoms are cough and shortness of breath. Computed tomography (CT) is the most important tool for establishing the diagnosis. Surgical repair is the treatment of choice in all cases to prevent complications.

We present a rare case of a symptomatic diaphragmatic hernia in a female patient who presented in old age with an unusual clinical presentation of chest pain and shortness of breath and improved completely after laparoscopic surgical repair.

Case Report

We report a case of 75-year-old female who came with 4 months history of respiratory difficulty and chest pain of 15 days duration. She was given symptomatic treatment and was diagnosed as a case of atrial fibrillation with fast ventricular rate. Inspite of taking full treatment, the patient's symptoms worsened, so was referred to the respiratory medicine department for further evaluation. There was no history of trauma, surgical intervention. There was no significant past medical history or family history of coronary artery disease, or airway diseases. She was asymptomatic before this episode since childhood. Chest pain was right-sided with severe intensity, not radiating; pressure quality increased with respiration, with difficulty in breathing, and relieved by standing and worsened on lying flat. There was no aggravation on walking. This was not associated with palpitations, dizziness, wheeze, or pedal oedema. Her general physical examination and systemic examination was normal. Her chest wall was non tender, lungs were having clear breath sounds bilaterally without any evidence of wheezing, rales, or rhonchi. She was investigated and all investigations were normal. CECT chest was suggestive of a large Morgagni hernia with defect in the right dome of diaphragm, with mild emphysematous changes and fissure thickening (as shown in image), large sliding hiatus hernia is also seen with bilateral mild pleural thickening. Adult onset diaphragmatic hernia is a rare condition with variable clinical manifestations. The majority of adult-onset diaphragmatic hernias are associated with trauma; but this patient denied history of any trauma – recent or past. The patient was evaluated by a surgeon and eventually underwent laparoscopic mesh repair of the diaphragmatic hernia. Her symptoms resolved on follow-up after surgical correction.

Discussion

Morgagni hernia (MH) is a congenital diaphragmatic hernia.

Professor, **Assistant Professor, ***PG Resident, Department of Respiratory Medicine, ESIC Medical College and Hospital, NIT-3, Faridabad - 121 001, Haryana.

Corresponding Author: Dr Ruchi Arora Sachdeva, Associate Professor, Department of Respiratory Medicine, ESIC Medical College and Hospital, NIT-3, Faridabad - 121 001, Haryana. Phone: 9999571169, E-mail: drruchiarorasachdevaesic@gmail.com.

It is rare and comprises only about 2% of all diaphragmatic hernias[3]. MH occurs due to an anteromedial diaphragmatic defect. Almost always, it occurs on the right side of the sternum (91%), which is the same side as in our patient; it occurs on the left side in only 5% of patients. Only 4% of the reported cases are bilateral.

The defect results from a fusion failure of the diaphragm with the costal arches[2-6]. Sanford *et al* reported that the average length of the diaphragmatic defect in the greatest dimension is 7.5 cm[6]. Patients can be asymptomatic most of the time which can results in delay in diagnosis. Only a few rare symptomatic adult cases have been described[7].

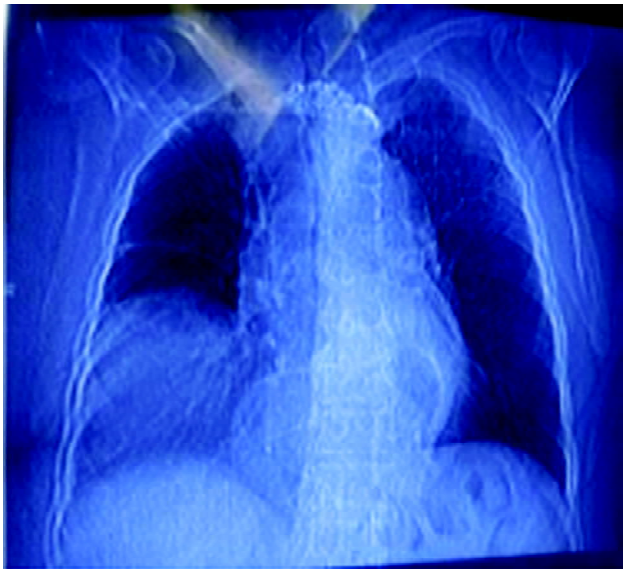

Fig. 1: *Chest X-ray PA view showing a homogeneous opacity in the right middle and lower zone.*

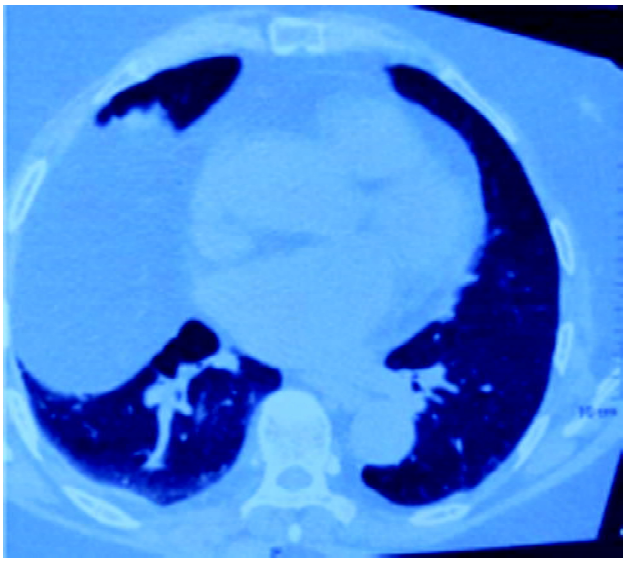

Fig. 2: *CT scan showing a right sided Morgagni hernia.*

Most patients present in childhood with respiratory issues, whereas in adults symptoms are nonspecific, respiratory or gastrointestinal symptoms or mostly asymptomatic which results in delay in diagnosis. In symptomatic cases, respiratory symptoms are the most common presenting complaints in about 34% of cases[6]. In some cases, symptoms include cough, dyspnoea, and chest pain. New-onset respiratory complaints in a formerly asymptomatic individual may be an early indication of progression of MH[8]. Abdominal pain can be due to incarceration or strangulation of the viscera, which are dreaded complications[9,10]. Pregnancy, trauma, obesity, chronic constipation, and chronic cough are common predisposing conditions contributing to the development of MH. Exercise and other types of exertion may also result in symptoms[11]. Women tend to present after the age of 50 years; men present earlier in life with complaints related to their hernia[8]. The most common contents of the hernia sac are abdominal visceral organs including the omentum, followed by the colon, stomach, small bowel, and part of the liver[2]. MH can be present on either side of the sternum; but, it is more commonly found on the right side. Most cases are asymptomatic. In symptomatic cases, the most common presenting symptoms are cough and shortness of breath. Li *et al* reported that the most common abdominal organs found in the hernia sac are the colon and omentum, and less frequently the small bowel, stomach, and liver[3]. The presence of a hernia sac is associated with better outcomes, whereas thoracic herniation of the liver is associated with worse outcomes. A similar case report was reported with chest pain by Mohamed *et al*[11]. In paediatric patients, it can be associated with other comorbid conditions, e.g., cardiac anomalies and major foetal defects; and MH has little effect on the outcome of the co-morbid diseases[12].

Computed tomography (CT) is the most important tool for establishing the diagnosis. Surgical repair is the treatment of choice in all cases to prevent complications[13]. The most feared complication of MH is strangulation and obstruction, rare complications including gastric volvulus with small intestine diverticulosis have been reported with MH[14]. Therefore, even if a patient is asymptomatic, surgical repair of MH is always indicated to prevent dreaded complications[3]. Surgical correction is the only established management for MH; however, because of the rarity of this pathology, there are currently no widely accepted guidelines on a standardised surgical technique in the literature[6]. A variety of surgical techniques are available include open abdominal approaches via laparotomy; open thoracic approaches via median sternotomy or thoracotomy; and minimally invasive techniques, including laparoscopy and thoracoscopy. There are various advantages and disadvantages associated with each approach in the repair of MH[6]. Minimally invasive surgery in laparoscopy carries

the shortest recovery time, offering almost immediate return to normal activities and diet by 3 days in a majority of cases and with a complication rate as low as 5%, which makes it the most favoured approach in uncomplicated cases. However, this method may prove suboptimal for complicated cases, because failure to reduce contents may necessitate open surgery[2]. Results of surgical repair are excellent.

Conclusion

Morgagni hernia (MH) is the rarest type of congenital diaphragmatic hernia. This is diagnosed predominantly in the first few hours of life or in the antenatal period. It is rare in adults, as it is mostly asymptomatic in adults and often detected incidentally. Symptomatic presentation in an adult is a rare event, which inspired us to report this rare case. Although rare, MH should be considered in the differential diagnosis of an adult with chest pain with respiratory distress after ruling-out other common causes.

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CASE REPORT

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A Complex Case of Renal Tertiary Hyperparathyroidism with a very Rare Brown Tumour of the Jaw Requiring Treatment with Initial Subtotal Parathyroidectomy followed by Maxillary Surgery and Haemodialysis

Indrajit Talapatra, Vijay Pothula***

Abstract

We describe here the case of a middle-aged woman who presented to the endocrine clinic in 2020 with chronic renal impairment and raised PTH (parathyroid hormone). The patient had seen an endocrinologist with anorexia nervosa and electrolyte abnormalities several years ago, a few times and was subsequently lost to follow-up. On this occasion her serum calcium was varying from normal to high, phosphate was normal, alkaline phosphatase high and PTH was high as well. Ultrasound of the neck suggested a radiologically U3 (indeterminate) nodule on the right lobe of thyroid and a possible parathyroid adenoma on the left which was not confirmed on sestamibi scan. The FNA (Fine needle Aspiration) of the right thyroid nodule suggested Papillary carcinoma. The patient also was found to have a Brown tumour of the maxilla, a very rare bone lesion secondary to renal hyper-parathyroidism. The patient initially underwent right haemithyroidectomy and subtotal parathyroidectomy with eventual completion left thyroidectomy. There was no improvement of this bone lesion despite lowering of PTH following subtotal parathyroidectomy and as such the patient required maxillary surgery one year later. The patiently remains currently on haemodialysis.

Key words: Renal, hyperparathyroidism, parathyroidectomy, Brown tumour, papillary carcinoma

Introduction

Tertiary hyperparathyroidism occurs when an excess of PTH is secreted by the parathyroid glands, after longstanding secondary hyperparathyroidism[1]. In secondary hyperparathyroidism (in chronic renal impairment or vitamin D deficiency), serum calcium is low or low-normal, phosphate is high, and PTH is high; and in tertiary hyperparathyroidism serum calcium, phosphate, and PTH are all raised. However, in renal hyperparathyroidism, the phosphaturia effect of PTH and FGF-23 (Fibroblast Growth Factor-23) sometimes may account for the serum phosphate level to remain normal[2]. In tertiary hyperparathyroidism, secondarily hyperplastic parathyroid glands of renal failure are no longer under secretory control of PTH by calcium, and therefore secrete more PTH and cause hypercalcaemia. The initial medical treatment with calcimimetics like Cinacalcet, vitamin D supplementation and low phosphate diet with phosphate binders, like calcium acetate or sevelamer (if serum phosphate is high) may not always be effective and the patient may require surgery in the form of subtotal parathyroidectomy or total parathyroidectomy with auto-transplantation[1]. Common indications for surgery for hyperparathyroidism in renal impairment include elevated levels of PTH, hypercalcaemia, very high phosphate (> 1.95 mmol/L), bone disease (osteitis fibrosa cystica), severe symptoms (pruritus, bone pain), and progressive ectopic calcification, and calciphylaxis[3]. Parathyroidectomy is shown to improve symptoms, bone and mineral metabolism, cardiovascular risk factors, and overall quality of life[4,5].

Case report

A 49-year-old woman was referred with chronic renal impairment and high PTH to the endocrine clinic in November 2020. She had a background history of anorexia nervosa (first seen by an endocrinologist in December 2009 with electrolyte abnormalities), osteoporosis, and had a left jaw swelling. The patient was under the care of the nephrology department at a tertiary hospital. Her medications at this point included Magnesium Oxide, Multivitamins, Alfacalcidol 1 mcg twice a week, Slow sodium tablets, Ferrous Fumarate, and Thiamine.

Her latest blood results showed serum urea 10.4 mmol/L (N: 2.5 - 7.8), creatinine 221 umol/L (N: 44 - 97), eGFR 21 mL/min, PTH 52.7 pmol/L (Normal: 2.0 - 9.3), adjusted calcium 2.50 mmol/L (N: 2.20 - 2.60), alkaline phosphatase 172 U/L (N: 30 - 130), phosphate 1.05 mmol/L (N: 0.8 - 1.45) vitamin D 50.4 nmol/L (N: 50 - 125) and Hb 111. The

**Professor, Department of Diabetes and Endocrinology, **Consultant, Department of ENT, Royal Albert Edward Infirmary, Wigan, WN1 2NN, UK.*

Corresponding Author: Dr Indrajit Talapatra, Professor, Department of Diabetes and Endocrinology, Royal Albert Edward Infirmary, Wigan, WN1 2NN, UK. Phone: 00447404429078, E-mail: indra_tala@hotmail.com.

diagnosis was renal tertiary hyperparathyroidism, the serum calcium was varying from normal to elevated levels. Her serum adjusted calcium was raised at 2.70 mmol/L, 2.79 mmol/L and 2.83 mmol/L a few months ago. Her serum phosphate remained normal, and her alkaline phosphatase was persistently raised from renal osteodystrophy.

The patient's ultrasound neck showed a hypoechoic lesion adjacent to the inferior pole of the left thyroid with increasing vascularity likely to be parathyroid adenoma and a well-defined heterogeneous predominantly hypoechoic nodule in the upper pole of right lobe of thyroid with internal calcifications with predominantly peripheral vascularity, thought to be radiologically U3 (indeterminate) in nature. The patient next underwent a sestamibi scan which however showed no parathyroid adenoma.

Her FNA of the right thyroid nodule performed by the ENT Consultant suggested histologically papillary carcinoma of thyroid. The decision following a multidisciplinary team meeting, was taken to consider a subtotal parathyroidectomy, given the diagnosis of renal hyperparathyroidism, together with right hemithyroidectomy. Histological study suggested parathyroid hyperplasia and papillary carcinoma with a lymph node metastasis and the patient thereafter needed complete left thyroidectomy.

The patient subsequently was managed on Levothyroxine and Calcichew D3, together with Alfacalcidol, which were later discontinued as her serum calcium was raised (elevated at 2.98 mmol/L). Her PTH continued to remain low, between 0.5 pmol/L to 2.0 pmol/L with the latest level being 1.2 pmol/L (hypoparathyroidism).

Her biopsy of the left maxillary swelling suggested Giant cell rich lesion (Brown tumour). Brown tumour is very rare in renal hyperparathyroidism and can sometimes regress with subtotal parathyroidectomy. In this case however, the patient required partial anterior maxillectomy with obturator placement 1 year later.

The patient has now been receiving haemodialysis twice a week at a tertiary hospital as the eGFR was varying between 9 - 11 mL/min and the creatinine between 438 to 476 umol/L.

Discussion

Renal hyperparathyroidism is associated with increased risks of fractures, cardiovascular disease, and death. It can be treated medically, but surgical parathyroidectomy is an option when medical treatment is not helpful or when associated with complications like renal bone mineral disease and calciphylaxis or calcific uraemic arteriolopathy[6]. Renal secondary hyperparathyroidism results from low levels of vitamin D, inability to activate vitamin D, low serum calcium, and diminished renal excretion of phosphate[7]. Continued excess PTH secretion will ultimately cause raised calcium levels. Subtotal and total parathyroidectomy with auto-transplantation are recognised surgical options, in case of unsuccessful medical treatment, followed by renal replacement therapy[7,8]. Surgery, is usually the mainstay of treatment in tertiary hyperparathyroidism in very advanced kidney failure[9].

Brown tumour of hyperparathyroidism caused by increased osteoclastic activity and fibroblastic proliferation (Osteitis Fibrosa Cystica) is histologically a Giant cell rich lesion[10]. Brown tumours in longstanding hyperparathyroidism are seen in 3 - 4% of primary hyperparathyroidism and in 1.5% of secondary hyperparathyroidism. They involve the ribs, clavicles, pelvis, femur, and jaw bones – with the mandible more commonly affected than the maxilla[11]. No exact statistical data is available of the association of this rare tumour with tertiary hyperparathyroidism. There was no response to subtotal parathyroidectomy with lowering of PTH and the patient required maxillary surgery.

The patient is currently on Levothyroxine, with a target to keep TSH suppressed (N: 0.35 - 5.5 mU/L) and is also managed in accordance with the the six goals of management of chronic hypoparathyroidism[12]:-

- Ensure that the patient does not have the symptoms of hypocalcaemia.
- Improve the patient's QoL (Quality of Life).
- Maintain serum calcium levels in the low-normal range.
- Keep serum phosphate within the normal range.
- Keep total calcium-phosphate product under 4.4 $mmol^2/l^2$ (55 mg^2/dL^2).
- Prevent hypercalciuria.

The patient remains on haemodialysis with future option of renal transplantation.

Conclusion

This is an interesting case; thyroid cancer was detected while investigating for raised PTH in renal impairment (requiring total thyroidectomy), a rare Brown tumour of jaw was associated with no improvement after lowering of PTH following subtotal parathyroidectomy, requiring maxillary surgery.

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for the surgery of the Brown tumour of the maxilla.

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CASE REPORT

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Hypokalaemic Periodic Paralysis with Renal Tubular Acidosis in Patients with Primary Sjögren's Syndrome Presenting as Quadriparesis and Recurrent Paraparesis

Mukesh Kumar Sarna, Rishabh Parakh**, Sarthak Shah**, Mansi Setia**, Manish Pahadia***, Sudha Sarna*****

Key words: Sjögren's syndrome, renal tubular acidosis (RTA), hypokalaemia.

Introduction

Sjögren's syndrome is a slow-progressing autoimmune disorder that involves exocrine glands, mostly lacrimal and salivary glands, resulting in impaired secretion of these glands, termed as sicca symptoms which is a combination of dry eyes (keratoconjuctivitis) and dry mouth (xerostomia)[1]. In addition to salivary glands and lacrimal glands involvement, Sjögren's syndrome affects other exocrine glands and organs such as the kidneys and liver. Primary Sjögren's syndrome commonly involves kidneys leading to Tubulointerstitial nephritis, type 1 Renal Tubular Acidosis (RTA), Fanconi syndrome, and Glomerulonephritis. Renal involvement is seen in 16% to 30% of patient. Type 1 or distal Renal Tubular Acidosis (RTA) is the most common presentation. Sjögren's syndrome is more commonly seen in middle-aged women with a female to male preponderance ratio of 9:1[2]. Hypokalaemic paralysis is the initial symptom in only 7% of patients with Sjögren's syndrome[2]. A diagnosis of hypokalaemic paralysis should be considered in a patient with hypokalaemia who presents with quick-onset neurological symptoms[3].

We hereby present two cases of hypokalaemic periodic paralysis presenting as quadriparesis and paraparesis, with distal renal tubular acidosis, which were later diagnosed to be secondary to Sjögren's syndrome. Informed consent was taken from each of these patients to present these as case reports.

Case report 1

A 35-year-old female presented to the medicine OPD with a 3-days history of sudden onset bilateral lower limb weakness involving proximal and distal muscles, swelling, and tingling sensation in bilateral lower limbs. Patient had mild dyspnoea, fever with chills and rigors, dry cough, history of dryness of mouth and foreign body sensation in eyes since 1 year. She had no history of altered sensorium, seizures, and vomiting, gastrointestinal symptoms like diarrhoea, joint pain, rashes and alopecia, use of steroids or laxative abuse, or herbal medicines in the past. She had no history of diabetes, hypertension, asthma, or tuberculosis. She did not have bladder or bowel involvement. She had three episodes of sudden-onset bilateral lower limb weakness, around one month back, around one year back, and around one- and half-years back; all improved with treatment.

On physical examination, patient was well-oriented to time, place, and person; the cranial nerves examination was normal; all deep tendon reflexes were diminished; the muscle power of both lower limbs was 1/5 and the upper limbs was 5/5 by Lovett's scale; and the rest of the CNS examination was normal. Other organ examinations, like gastrointestinal, respiratory, and cardiovascular, were normal. Her tongue was dry, and infralingual salivary pooling was present. Her vital signs on admission were temperature 98.9° F, heart rate 90 beats per minute, respiratory rate 20 breaths per minute, oxygen saturation 97% at room air, capillary blood glucose 110 mg/dL, and blood pressure was 106/70 mmHg. Routine investigations were sent, which are mentioned in Table I below. Serum potassium level was 2.1 mEq/L, and ABG revealed normal anion gap metabolic acidosis with urinary pH of 5.7. As her history of dryness in the mouth pointed towards an autoimmune disorder, an anti-nuclear antibody by Immunofluorescent technique (ANA by IFA) was sent, which came positive in a 1:80 titre with speckled pattern grade one+, ANA profile was sent to confirm, which revealed positive SS-A and SS-B antibodies, which are positive in Sjögren's syndrome. An ophthalmologist's opinion was taken to further investigate Sjögren's syndrome. Schirmer's test was conclusive in favour of Sjögren's syndrome, her left eyes showed no signs of tears, (i.e., a very severe form of dry eye), and the right

**Professor and Unit Head, **Resident, ***Professor, Department of General Medicine, ****Professor and Head, Department of Palliative Medicine, Mahatma Gandhi Medical College and Hospital, Jaipur - 302 022, Rajasthan.*
Corresponding Author: Dr Sudha Sarna, Professor and Head, Department of Palliative Medicine, Mahatma Gandhi Medical College and Hospital, Jaipur - 302 022, Rajasthan. Phone: 9829117488, E-mail: mukeshsarna@gmail.com.

eye revealed moderate dry eye (6 mm). Her TFBT (Tear Film Break-Up Test) revealed dryness in both eyes; all were conclusive in favour of Sjögren's syndrome. With the help of history, examination and lab results, diagnosis of distal RTA (dRTA) with primary Sjögren's syndrome causing hypokalaemic periodic paralysis was made. For hypokalaemia, the patient was treated with intravenous potassium chloride and sodium bicarbonate. Further, hydroxychloroquine was started with a dose of 200 mg once daily, and steroids with a dose of 1 mg/kg body weight were initiated. The patient responded to the treatment, and her clinical profile showed improvement. The patient was discharged as her symptoms improved. The patient has been on regular follow-up for the last 5 months, is asymptomatic, and is on a tapering dose of steroids and alkali supplements.

Case report 2

A 23-year-old female student, a known case of hypothyroidism, resident of Bikaner, Rajasthan, developed sudden onset weakness in all four limbs while she was walking towards her house. She fell down and was taken to a nearby hospital where basic investigations were done. The weakness was symmetrical and non-progressive in nature. Arterial blood gas analysis was suggestive of severe metabolic acidosis with hypokalaemia (S. potassium: 1.5 mEq/L). Supportive treatment was given, and she was referred to a higher centre. On the way in the ambulance, she had an episode of asystole, for which she received cardiopulmonary resuscitation (CPR). The return of spontaneous circulation was achieved. She was intubated for better resuscitation efforts and brought to our centre. The patient was immediately admitted to the ICU, and all baseline investigations were sent, which are mentioned in Table I.

Intravenous potassium with magnesium were started, along with supportive treatment. The patient was intubated and was on FiO_2 40%. She had deranged sensorium so MRI of the brain was done. It was suggestive of hypoxic ischaemic encephalopathy. Besides this, the patient had recurrent hypokalaemia. Even after correction, potassium levels were below normal levels. There was no plausible cause for recurrent hypokalaemia. Distal RTA was kept as a differential as ABG was suggestive of metabolic acidosis. Anti-nuclear antibody (ANA) levels was sent as dRTA is found to be associated with Sjögren's syndrome. The ANA report came out positive with a titre of 1:120 speckled titre one positive. Thereby, an ANA profile was sent, which was positive for anti-Ro and anti-La antibodies. To confirm the diagnosis of Sjögren's syndrome, ophthalmologist's opinion was taken. Schirmer's test was performed, which was conclusive in favour of Sjögren's. Her right eye showed no signs of tears, (i.e., a very severe form of dry eye), and the left eye revealed severe dry eye (4 mm). Then patient was diagnosed as having primary Sjögren's syndrome with distal renal tublar acidosis causing hypokalaemic periodic paralysis, though she did not have any sicca symptoms in the past suggestive Sjögren's syndrome. Patient might have had cardiac arrest because of hypokalaemia only. Patient's attendants took discharge on request and did not come back for a follow-up visit; so further work-up could not be done.

Table I:

Lab tests	Patient 1	Patient 2
Haemogram (Hb/WBC/Plt)	10.5/7.7/84 k	10.2/12/336 k
ABG (pH, PCO2, HCO3, PO2, Lactate)	7.367, 28.1, 17.8, 1.2	7.1, 21.1, 10, 90, 3
Urine pH	5.7	6.0
ESR	90	43
Serum electrolytes ($Na^+/K^+/Cl$)	Day 1 - 141/2.1/113 Day 3 - 135/3.02/111 Day 5 - 136/3.15/110 Day 7 - 141/4.31/122	141/1.3/113
ANA by IFA	Positive 1:80 Speckled	Positive 1:120 Speckled
PBF	Microcytic hypochromic with anisopoikilocytosis in form of target cells	Microcytic hypochromic with anisopoikilocytosis
Viral markers (HBSAG, HIV 1&2, HCV)	Negative	Negative
LFT	WNL	WNL
RFT (blood urea, S. creatinine, S. uric acid)	19.6/0.8/3.1	57.3/2.8/9.8
S. iron	23.5	23.5
TSH	1.367	1.367
Urine R/E	WNL	WNL
ANA profile	SS-A/R060 KD SS-A/R052 KD SS-B/LA	SS-A/R060 KD SS-A/R052 KD SS-B/LA

Discussion

Systemic autoimmune disease Sjögren's syndrome is characterised by a distinct combination of signs and symptoms that are mostly brought on by a cell-mediated autoimmunity towards exocrine glands. Approximately 30 to 40 per cent of patients with primary Sjögren's syndrome experience systemic symptoms. The kidney is the non-exocrine organ most frequently impacted by Sjögren's syndrome. Distal renal tubular acidosis (dRTA) is the most typical type of renal involvement in Sjögren's syndrome. It is typically asymptomatic and goes unnoticed in most cases. Interstitial nephritis follows. The most frequent electrolyte imbalance in dRTA patients is hypokalaemia[4].

Distal RTA can occur on its own or as a subsequent consequence of other illnesses like chronic hepatitis, autoimmune disorders, and transplant rejection. For

autoimmune illnesses, the connection between Sjögren's syndrome and distal RTA is well known. The possible mechanism causing distal RTA is the lack of an H±ATPase pump in intercalated cells in the collecting tubules caused by immune-mediated injury, which may be the cause of distal RTA in Sjögren's syndrome[5]. This decrease in secretion and subsequent retention of hydrogen ions causes an increase in potassium excretion in exchange for sodium reabsorption in the collecting tubules to maintain electroneutrality. The other mechanism is a dysfunctional H±ATPase pump that causes sodium loss and, in turn stimulates the action of the hormone angiotensin-aldosterone, which results in hypokalaemia.

In our case series, patient 1 presented with paraparesis and patient 2 presented with quadriparesis; both their investigations revealed significant hypokalaemia and metabolic acidosis as the causes of the presenting complaint, and primary Sjögren's was suspected. All suspected instances of primary Sjögren's syndrome should be tested for the presence of anti-SSA antibodies (antibodies against Sjögren's syndrome-related antigen A), which are present in two-thirds of patients. In the absence of anti-SSA antibodies, a minor salivary gland biopsy is often advised to confirm a diagnosis of primary Sjögren's syndrome. A helpful test to determine ocular dryness is Schirmer's test. The latest and new American College of Rheumatology/European League Against Rheumatism ACR/EULAR established a new set of classification criteria for primary SS in 2016 which is enlisted in the Table II, and the diagnosis requires a minimum score of 4[6,7]. Both our patients met the criteria with a score of 4 (Anti-SSA/Ro, Schirmer's test positive), hence our diagnosis was confirmed.

Primary SS is treated symptomatically. When a patient with hypokalaemia appears in an emergency situation, the goal is to reverse the severe hypokalaemia with intravenous potassium supplementation, which treats the underlying acidosis. For the majority of patients, long-term potassium supplementation may be necessary. Muscarinic agonists are suggested for the treatment of mouth dryness and, to a lesser extent, ocular dryness. A combination of corticosteroids and other immunosuppressive medication has been claimed to reduce the course of renal impairment (tubular defects) in Sjögren's syndrome[8,9]. A more individualised strategy is required to enhance long-term outcomes in patients with primary SS due to the heterogeneity in the aetiopathogenesis and clinical manifestation of the disease, as well as a variable response to clinical treatments.

A score >4 classifies a patient who meets the including criteria and does not have any of the exclusion criteria.

Table II: The ACR/EULAR classification criteria for Primary Sjögren's syndrome 2016[6,7].

Item	Weight/Score
Labial salivary gland with focal lymphocytic sialadentitis and focus score of > 1 foci/4 mm^3	3
Anti-SS-A/RO positive	3
Ocular staining score > 5 in at least one eye	1
Schirmer's test < 5 mm/5 minutes in at least one eye	1
Unstimulated whole saliva flow rate < 0.1 mL/minute	1

Conclusion

Clinically Sjögren's syndrome may present differently in different patients. Patients presenting with renal symptoms as recurrent hypokalaemia as the initial presentation in Sjögren's syndrome pose much difficulty in diagnosis. This example serves as a reminder of the need to maintain a high index of suspicion for Sjögren's syndrome in patients presenting with recurrent hypokalaemia, especially middle-aged females.

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