C O N T E N T S

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ORIGINAL ARTICLE

Deep Vein Thrombosis in Cancer Patients:
A Pilot Study from Eastern India

Madhuchanda Kar*, Digbijoy Chowdhury**, Sayan Das***

Abstract

Background: Patients with cancers are at higher risk of developing deep vein thrombosis (DVT) than others. This study determined the prevalence of DVT and analysed its association with disease subtypes and previously established risk factors. There are very few studies on DVT in cancer patients from our country and this is the first of its kind from the eastern part of India.

Methods: This retrospective cross-sectional study enrolled patients aged >18 years with cytologically or histopathologically confirmed malignancies. Medical history, demographic characteristics, comorbidities, cytological or histopathological subtypes of these cancers were noted at the presentation.

Results: Out of 147 patients, 11 (8.59%) had DVT and a female preponderance (72.7%) was noted. The mean age (years) of the patients with DVT was significantly lower than patients without DVT (48.64 versus 59.68; p = 0.005). A significant correlation between DVT and age of patients was observed (p = 0.045). Cancer patients < 55 years of age had DVT, that was 3.49 times more than prevalent in patients > 55 years of age (Odds ratio = 0.286; 95% CI: 0.079 - 1.035). Risk factors like gender, diabetes, and hypertension did not show any definite association with DVT prevalence (p > 0.05). Prevalence of DVT was highest amongst patients with underlying breast and gynaecologic cancers (36.4%, each). The subtypes of cancer did not show any significant association with occurrence of DVT (p > 0.05).

Conclusion: These are the observations about the prevalence of DVT in cancer patients in this eastern part of the country and their correlation with age of the patients. However, cancer site, subtype, and standard comorbidities did not have any statistically significant correlation with the prevalence of DVT. In this study DVT was mostly observed in younger female patients who had breast and gynaecological cancers.

Key words: Cancer subtype, chemotherapy, deep vein thrombosis, gynaecological cancers.

Introduction

Globally, cancer patients are at higher risk of developing deep venous thrombosis (DVT) and subsequent serious venous thromboembolism (VTE). This risk is 4 to 6 times higher in the cancer population as compared to non-cancer cohorts[1,2]. The repercussions of malignancy on venous stasis, imbalance of blood coagulants, and vessel wall damage substantially contribute to the alterations in normal blood flow (rheology),thereby increasing thrombus formation[3].

Development of thrombosis in cancer patients results in their poor prognosis and has been associated with increased mortality and morbidity[7,8]. Prevalence of DVT in cancer patients in India is not uncommon and there is an underestimation of these risks as well as low levels of clinical awareness. Limited use of thrombo-prophylaxis with high fatality from VTE has made DVT a worldwide cause of concern for mortality of cancer patients. But there is a scarcity of published literature related to the prevalence of DVT in cancer patients in India ,specially the lack of available

data from this part of the country. Therefore, the present retrospective cross-sectional study was designed to determine the prevalence of DVT in cancer patients at presentation in this tertiary referral centre of Eastern India. This study will help us to be aware of this very important and preventable complication of cancer that is not uncommon, but often overlooked even in this part of the subcontinent.

Methods

A retrospective cross-sectional study was conducted in a tertiary care multispeciality centre of Eastern India during the period of May 2017 to May 2018. The study protocol was approved by the Institutional Ethics committee and was in accordance with the principles of the Declaration of Helsinki. Patients >18 years of age with cytologically or histopathologically confirmed malignancies were enrolled in the study. The patients already on any anticoagulant or antiplatelet drugs were excluded from the study.

*Clinical Director, Department of Oncologh, **Ex-Senior Registrar, Department of Medicine, ***Interventional Radiologist, Peerless Hospitex Hospital and Research Center Limited, 360 Panchasayar, Kolkata - 700 094.
Corresponding Author: Dr Madhuchandakar, Clinical Director, Department of Oncology, Hospitex Hospital and Research Center Limited, 360 Panchasayar, Kolkata - 700 094. Tel: 9830155915, E-mail: madhuchandakar@yahoo.com.

The medical records were retrospectively reviewed for detailed medical history, demographic profile, site of cancer, presence of comorbidities like hypertension and diabetes mellitus, co-prescription of drugs, cytological or histopathological confirmation of malignancies and their subtypes in all the patients included in this study. Diagnosis of DVT was suspected if patients showed positive clinical signs and symptoms like localised oedema, pain, warmth, reddish-blue discoloration over limbs (especially when unilateral) and diagnosis was confirmed with Doppler ultrasonography.

Statistical analysis of data was done using SPSS version 23.0. Categorical data were expressed as number (percentages) and continuous data were expressed as mean (standard deviation (SD). Corelation between different clinicopathological parameters and DVT prevalence was assessed using Pearson's chi-square test. Fisher's exact test was used for finding the correlation between risk factors and DVT. The p value< 0.05 was considered as statistically significant.

Results

Of the total 148 patients with confirmed malignancies and whose lower limb Doppler USG results were available (Table I), 11 patients (8.59%) were diagnosed with the presence of DVT and 117 patients (91.41%) were without DVT. The mean (SD) age of the study population was 58.72 years with 70 (54.6%) females and 58 (45.4%) males. The mean (SD) age (years) of the patients with DVT was significantly lower as compared to patients without DVT [48.64 (8.62)] versus [59.68 (12.45)]; p = 0.005. Although the prevalence rate of DVT in females (n = 8, 72.7%) was higher than in males (n = 3, 27.3%), this was not statistically significant (p = 0.2). A significant correlation between DVT prevalence and age of patients was observed (p = 0.045). Interestingly, it was found that DVT was 3.49 times more common in cancer patients <55 years of age (Odds ratio = 0.286; 95% CI: 0.079 - 1.035). Amongst patients with DVT, 36.4% of patients had hypertension and 27.3% had diabetes as comorbidities. Whereas, in patients without DVT, 16.2% of patients had hypertension and 17.9% had diabetes. However, this corelation with standard comorbidities was not statistically significant.

As tabulated in Table II, the highest DVT prevalence rate was observed in patients with breast cancers (36.4%) and gynaecologic cancers (36.4%) followed by patients with digestive tract cancers (18.2%) and lung cancers (9.1%). However, the type of cancer did not show any statistically significant association with DVT prevalence ($\div2$ =12.911; p = 0.16). Morphological subtyping of cancer also did not

show any significant association with DVT prevalence (p = 0.3).

Table I: Correlation of deep vein thrombosis (DVT) with clinical characteristics in cancer patients.

Parameters	Patients with DVT (n = 11)	Patients without DVT (n = 117)	Total (n = 128)	Odds Ratio (95% CI)	p-value
Age (years), mean (SD)	48.64 (8.62)	59.68 (12.45)	58.72 (12.54)	–	0.005
≤ 55 years	07 (63.6)	39 (33.3)	46 (36.2)	0.286	0.045*
> 55 years	04 (36.4)	78 (66.7)	82 (63.8)	(0.079 - 1.035)	
Sex					
Male	03 (27.3)	55 (47.0)	58 (45.4)	0.423	0.2
Female	08 (72.7)	62 (53.0)	70 (54.6)	(0.107 - 1.673)	
Hypertension					
Present	04 (36.4)	19 (16.2)	23 (18.3)	2.947	0.09
Absent	07 (63.6)	98 (83.8)	105 (81.7)	(0.785 - 11.067)	
Diabetes					
Present	03 (27.3)	21 (17.9)	24 (18.3)	1.714	0.45
Absent	08 (72.7)	96 (82.1)	104 (81.7)	(0.419 - 7.010)	

*Data shown as n (%), unless otherwise specified.*Significance with p < 0.05*

Table II: Correlation of deep vein thrombosis (DVT) with cancer-related parameters among the study population.

Parameters	Patients with DVT (n = 11)	Patients without DVT (n = 117)	Total (n = 128)	Odds ratio (95% CI)/ $\div2$ test value	p-value
Type of cancer					
Lung	1 (9.1)	6 (5.6)	6 (5.9)	$\div2$ = 12.911	0.16
Brain	0 (0.0)	1 (0.9)	1 (0.8)		
Breast	4 (36.4)	18 (16.7)	22 (18.5)		
Digestive	2 (18.2)	44 (40.7)	46 (38.7)		
Genitourinary	0 (0.0)	8 (7.4)	7 (6.7)		
Gynaecologic	4 (36.4)	10 (9.3)	14 (11.8)		
Haematopoietic	0 (0.0)	12 (11.1)	11 (10.1)		
Mouth	0 (0.0)	4 (3.7)	4 (3.4)		
Endocrine	0 (0.0)	2 (1.9)	2 (1.7)		
Others	0 (0.0)	3 (2.8)	3 (2.5)		
Morphology					
Adenocarcinoma	03 (100)	17 (73.9)	20 (71.4)	0.850	0.3
Squamous cell carcinoma	0 (0.0)	06 (26.1)	06 (28.6)	(0.707 - 1.022)	

Data shown as n (%); C: Chemotherapy; S: Surgery; R: Radiotherapy; Others: germ cell, pyriform sinus and sarcoma.

Discussion

The present retrospective cross-sectional study evaluated the prevalence of DVT in cancer patients registered with

this tertiary referral centre in eastern India and assessed the association of DVT with clinical characteristics, sites of malignancy, histopathological subtypes and comorbidities. A significant association between DVT prevalence and age of the patients was observed (p = 0.045). Although elderly age is in general risk factor for DVT, but interestingly in our study, younger patients (<55 years) had a higher prevalence. That maybe due to higher prevalence of breast and gynaecologic malignancies which are common in this particular age group. Gender, comorbidities like diabetes and hypertension did not show any statistically significant association with DVT. The site of cancer or histo-morphology also did not show any significant association with DVT prevalence in the present study.

A study by Lee et al has reported malignancy as a risk factor in 31% of Indian patients with DVT[9]. A recent study by Kamerkar et al showed that 7% of patients with DVT had malignancy as a predisposing factor and genitourinary cancers had the highest incidence of DVT[10]. Interestingly, our study showed a higher prevalence rate of DVT than that reported in earlier studies[7,10]. The present study reported 8.59% incidence of DVT in cancer patients with a mean age of 48.64 years and female (72.7%) preponderance. The mean age of cancer patients with DVT was significantly lower as compared to cancer patients without DVT (p = 0.005). This might be due to a relatively small number of cancer patients with DVT in this study than those without DVT (11 versus 117). A recent study by Fekri et al reported a DVT prevalence rate of 19.9% in cancer patients of which 21.2% were in males and 18.2% in females and there was no statistically significant correlation between gender and DVT prevalence (P = 0.409)[11]. Similarly, the present study also reported no significant relationship between gender and DVT prevalence (p = 0.2). However, female preponderance observed in the present study is in concordance with the study done by Dutia et al[13].

In the present study, a significant correlation between DVT prevalence and age of patients was observed (p = 0.045). Cancer patients with < 55 years of age were 3.49 times more prone to develop DVT than patients > 55 years of age (Odds ratio = 0.286; 95% CI: 0.079 - 1.035). The observations of a study done by Fekri et al showed that the patients between 40 to 65 and over 65 years of age had the highest DVT prevalence rate (21.6% and 20.1%, respectively); however, there was no significant correlation between age and DVT prevalence (p = 0.459) in that study also. Several other previous studies, have reported advanced age as an independent risk factor for VTE in hospitalised patients with cancer[14,15]. However, 2 retrospective studies did not find that advanced age was associated with the risk of DVT[13,16]. The variations in these observations in different studies can be attributed to the heterogeneity of different population

of cancer patients as far as size and timelines of their presentations were concerned.

This study reported no significant association between site of cancer or pathological subtype with DVT prevalence. DVT prevalence rate was highest in patients with breast cancers (36.4%) and gynaecologic cancers (36.4%) followed by patients with digestive tract cancers (18.2%) and lung cancers (9.1%). A study by Fekri et al showed that the DVT prevalence differs based on various pathological subtypes. Small cell carcinoma (42.5%), adenocarcinoma (26.9%), and squamous cell carcinoma (23.1%) were the malignancies with highest rates of DVT. Other previous studies have reported stronger association of DVT risk with adenocarcinoma than squamous cell carcinoma[7,17]. The present study did not show any statistical corelation with histopathological subtype. This might be due to the smaller size of the patient population in the current study and their different timelines of presentation.

This study was a pilot study done with limited number of patients. The retrospective design of the study also limited the confirmation of the effect of risk factors on the prevalence of DVT. There were also biases of referral. Only Doppler USG was used to diagnose DVT and no further confirmatory methods were used.

Conclusion

This is a pilot study on DVT in different types of cancers, and the first of its kind in eastern India. Observations in the present study revealed 8.59% incidence of DVT in cancer patients. Interestingly, a significant association between age and DVT prevalence was observed. It reiterates that DVT is prevalent even in relatively younger populations with cancer, especially in breast and gynaecological malignancies. We should pro-actively search for it, irrespective of age, even in the absence of symptoms and comorbidities. In our study, other cancer-related parameters like site of disease, histological subtype or co-morbidities did not reveal any statistical corelation with the risk of DVT.

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Prevalence and Predictors of Thyroid Dysfunction in Human Immunodeficiency Virus (HIV)-Infected Individuals

Pulin Kumar Gupta, Rajesh Satyapal Taneja*, Lohit Chauhan**, Sharwani Lal Vijayshri***, Saurabh Tyagi**,
Ramesh Chand Meena**, Anahita Gupta****, Manita Khatak***

Abstract

Introduction: Acquired immunodeficiency syndrome (AIDS) is a chronic multisystem disease also affecting the neuroendocrine system including thyroidal endocrine axis.

Methods: It was a cross-sectional, observational study, done in 100 HIV-infected individuals. The cases were divided into sub group A (50 patients on anti retroviral therapy (ART)) and sub group B (50 treatment naïve patients not on ART). 50 age and sex matched healthy controls were also recruited.

Results: Amongst 100 cases the overall prevalence of thyroid dysfunction was 45% [70% in sub group A versus 20% in sub group B (p < 0.001)] as compared to 9.4% in controls. Overt hypothyroidism was found in 10% cases [14% in group A vs 6% in group B (p < 0.05)]. Subclinical hypothyroidism (SCH) was the most prevalent condition seen in 30% of our cases [50% in group A and 10% in group B (p < 0.0001)] and 6% of controls. The mean TSH was significantly higher in cases as compared to controls (5.12 ± 1.80 µIU/L vs 3.81 ± 1.12 µIU/L) (P < 0.001) and in gp A as compared to gp B (5.81 ± 1.75 µIU/L vs 4.42 ± 1.59 µIU/L) (P < 0.005). Similarly FT_3 and FT_4 were significantly lower in cases as compared to controls (P < 0.001) and in gp A as compared to gp B (P < 0.01). Stepwise linear regression revealed HIV infection per se along with longer duration of disease, low CD4 cell counts, exposure to ART, longer duration of ART, male sex and past history of tuberculosis as strongest predictors of thyroid dysfunction in HIV-infected these individuals.

Conclusion: Thyroid disorders (specially subclinical hypothyroidism) is a very common but under-reported entity in patients with HIV/AIDS and virus per se along with ART seems to be the biggest factor affecting thyroid endocrinopathy.

Keywords: CD4count, HIV, subclinical hypothyroidism.

Introduction

Acquired immunodeficiency syndrome (AIDS) is a chronic multisystem disease and neuroendocrine system is no exception to that. Endocrine changes in the form of thyroidal, adrenal, gonadal, bone, and metabolic dysfunctions have all been reported in both early and late stages of HIV infection[1]. Alterations in endocrine function may be due to the possible relationship between the immune and endocrine systems, direct involvement of the gland by virus itself, secondary endocrine dysfunction due to indirect effect of cytokines, opportunistic infections or highly active anti-retroviral therapy (HAART)[2]. In the last two decades, the advent of potent and efficacious HAART has given rise to increased life expectancy and hence higher incidence of chronic diseases including endocrinopathies in HIV-infected individuals[3].

Amongst all endocrinopathies in people with HIV/AIDS, thyroid dysfunction is probably the commonest but least reported and various studies have reported it to be in the range of 10 - 15% out of which 1 - 2% manifest overt thyroid disease and subclinical thyroid disorders have been reported in 10 - 14%[4]. The association between thyroid abnormalities and HIV is well documented in western literature. However, there is a paucity of data regarding the same in Indian HIV-infected population which makes it difficult to make general recommendations about hormone testing and replacement therapy in these individuals. Apart from that, even though studies have reported a very high prevalence of thyroid abnormalities like subclinical hypothyroidism in individuals with HIV/AIDS but whether it is due to HIV itself or due to HAART is still not clear. This cross-sectional observational study was undertaken to determine the prevalence and the pattern of thyroid abnormalities in HIV-infected individuals and their association with various disease AIDS related factors including duration and type of antiretroviral therapy and level of immunodeficiency, i.e., CD4 cell counts.

Material and methods

It was a cross-sectional observational study done over a span of one year in the department of medicine and

*Professor, **Resident, ***SMO, Department of Medicine, ABVIMS, Dr Ram Manohar Lohia Hospital, Kharak Singh Marg, New Delhi - 110 001; ****SMO, Department of Medicine, BSES, New Delhi.
Corresponding Author: Dr Pulin Kumar Gupta, Professor, Department of Medicine, ABVIMS, Dr Ram Manohar Lohia Hospital, Kharak Singh Marg, New Delhi - 110 001. Tel: 9899748321, E-mail: guptapulin@yahoo.com.

antiretroviral therapy (ART) centre at PGIMER, Dr RML Hospital after approval from the institutional ethical committee. The cases were 100 HIV-infected individuals, out of which 50 were on HAART (subgroup A) and 50 were HAART naïve, i.e., not on any anti-retroviral therapy (subgroup B). 50 age and sex-matched healthy relatives of cases volunteers were recruited as controls.

All cases with history of medical or surgical thyroid-related disorders (including use of thyroid hormones) any time in the past or with history of intake of drugs known to affect thyroid physiology, e.g., amiodarone, lithium, PAS, iodine, etc., were excluded. Similarly, all cases with past history of any opportunistic infections within the last one year or pre-xisting cardiac, renal, or liver related disorder or delivery/ lactation within the last six months was also excluded.

A thorough history and physical examination including that of the thyroid glands was done. After an overnight fasting 10 ml venous blood sample was collected for estimation of routine baseline investigations and thyroid function tests including FT3/FT4/TSH and anti-TPO antibody levels. Chemiluminescent Enzyme Immunoassay (CLEIA) using VITROS immunodiagnostic kit was used for estimation of FT3, FT4 and anti-TPO levels. Felikrimunometric immunoassay technique was used to measure serum TSH levels.

BECTON-DICKINSON FACS flow cytometer was used to obtain CD4-cell counts.

The standard normal values of the thyroid function tests were taken as per the reference range in the department of biochemistry of our institution.

Thyroid stimulating hormone (TSH): 0.5 - 5.0 mIu/ml

Free T3 (FT3): 2.0 - 4.4 pg/ml

Free T4 (FT4): 0.7 - 2.0 ng/dl

Anti-thyroid peroxidase antibody (ANTI-TPOAb): Up to 50 IU/ml.

Definitions used in the study

- Overt hypothyroidism: raised TSH (> 10 mIu/ml) and low FT4 levels.

- Subclinical hypothyroidism: raised TSH (5.0 - 10 mIu/ ml) and normal FT4 or FT3 levels.

- Overt hyperthyroidism: low TSH (< 0.5 mIu/ml) and raised levels of FT4 or FT3

- Subclinical hyperthyroidism: low TSH (< 0.5 mIu/ml) and normal FT4 or FT3 levels.

- Isolated low FT4: low FT4 levels with normal TSH and FT3 levels.

Statistical analysis

The analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric tests were used.

Quantitative variables were compared using Independent T test/Mann Whitney test (for non-parametric data) to compare between cases and controls. Qualitative variables were compared using Chi-Square test/Fisher's exact test. Pearson correlation co-efficient was used to find out the association between thyroid function tests with the duration of disease and HAART. P value of < 0.05 was considered statistically significant.

Results

A total of 100 HIV-infected individuals (cases) and 50 age and sex-matched healthy volunteers (controls) were enrolled in the study. The cases were further subdivided into subgroup A (50 cases on HAART) and subgroup B (50 cases not on HAART, i.e., treatment naïve). All subjects in subgroup A were on a combination of Tenofovir, Lamivudine and Efavirenz for at least two years. Amongst all subjects 60% were males and 30% were females. Maximum numbers of subjects (36%) were between 21 - 30 years of age group. The most common mode of transmission amongst all cases was heterosexual (77%) followed by homosexuality (3%), blood transfusion (4%), IV drug abuse (6%), and no cause was found in 10% of individuals and routine laboratory measurement were comparable amongst cases and controls.

Three out of every four cases complained of symptoms of easy fatiguability, lethargy, dry skin, irritability, and cold sensitivity; and females were significantly more symptomatic than males. However, sexual dysfunction was reported more by men (> 30%). None of the cases had clinically palpable goitre or any swelling in the neck or bruit. The mean CD4 cell counts of all the cases was 308.11/mm^3 [(292.44/mm^3 in subgroup A and 323.88/mm^3 in subgroup B). p < 0.005]. The profile of thyroid abnormalities in cases and controls was as under (Table I).

45% of cases as compared to 9.4% of controls had some thyroid dysfunction and the difference was statistically significant (p < 0.001). Similarly, overt or subclinical hypothyroidism was found significantly more in cases as compared to controls, i.e., 10% vs 3% and 30% vs 6% respectively. Similarly cases in subgroup A (cases on HAART) had significantly higher prevalence of overt and subclinical

hypothyroidism as compared to cases in subgroup B (i.e., treatment naïve cases) (14% vs 6% and 50% vs 10% respectively) and the difference was statistically significant. The anti-TPO antibody was found to be positive in five cases in subgroup A (10%) and 7 cases in subgroup B (14%). Two cases in subgroup A with anti-TPO positivity were found to have overt hypothyroidism and rest three had normal thyroid profile. The mean value of thyroid function tests amongst cases and controls has been tabulated in Table II.

Table I: Prevalence of thyroid abnormalities in cases (including subgroups) and controls.

	All Cases	Controls	Statistical difference	Subgroup A	Subgroup B	Statistical difference
Overall Thyroid dysfunctions	45%	9.4%	p < 0.001	70%	20%	p < 0. 05
Overt hypothyroidism	10%	3%	p < 0.005	14%	6%	p < 0.05
Subclinical hypothyroidism	30%	6%	p < 0.0001	50%	10%	p < 0.05
Isolated low FT_4	5%	0.3%	p < 0.05	6%	4%	p < 0.01
Subclinical/overt hyperthyroidism	0%	0%		0%	0%	
Anti TPO positivity	12%	8.9%		10%	14%	

Table II: Profile of thyroid function test in cases (including subgroups) and controls.

Mean values	Cases (n = 100)	Controls (n = 50)	P value (cases vs controls)	Subgroup A (n = 50)	Subgroup B (n = 50)	P value (GP A vs GP B)
TSH (µIU/L)	5.12 ± 1.80	3.81 ± 1.12	< 0.001	5.81 ± 1.75	4.42 ± 1.59	< 0.001
FT_3 (pg/ml)	2.38 ± 0.63	3.37 ± 0.73	< 0.01	2.62 ± 0.74	2.86 ± 0.77	< 0.04
Mean FT_4 (ng/ml)	1.26 ± 0.48	1.45 ± 0.43	< 0.05	1.18 ± 0.36	1.34 ± 0.56	< 0.01
Mean Anti TPO (IU/ml)	26.44 ± 6.92	28.86 ± 7.48	< 0.11	24.78 ± 5.48	28.13 ± 6.58	< 0.09

The mean value of TSH amongst cases was significantly higher than in controls [5.12 mIU/ml vs 3.81 mIU/ml (p < 0.001)]. Likewise mean FT3 and FT4 levels were significantly lower in cases as compared to controls implying that infection with HIV itself is associated with higher TSH (released from pituitary) and lower FT3/FT4 levels. Similarly, mean TSH was significantly higher in subgroup A as compared to subgroup B and likewise levels of FT3/FT4 were lower in subgroup A as compared to subgroup B [5.81 vs 4.42 mIU/ml (p < 0.001)] implying that HAART by itself may have a significant impact on the levels of thyroid hormones. The levels of TSH were significantly higher and FT3/FT4 were significantly low in subgroup B as compared

to controls meaning that HIV by itself (without the added impact of HAART) has a profound effect on the causation of hypothyroidism, meaning that HIV per se may have a direct suppressive effect on hormone production by thyroid gland (FT3 and FT4) and since FT3/FT4 have a feedback mechanism effect on the release of TSH from pituitary so there is a concomitant increase in the levels of TSH in these cases. Inspite of the so high prevalence of thyroid dysfunction and subclinical hypothyroidism reaching upto 45% and 30% respectively, only 12% of the cases had high anti TPO levels (10% in subgroup A and 14% in subgroup B), meaning that thyroid dysfunction in all cases and specially cases in subgroup A were more not because of immune phenomenon virus-induced thyroid destruction or effect of HAART itself on thyroid or pituitary.

Amongst cases in sub group A with thyroid dysfunction the mean duration of HAART therapy was 42.76 ± 11.23 months as compared to 36.77 ± 8.21 months in in those with normal thyroid functions (p < 0.05). It was found that there was a direct correlation between the duration of HAART and the levels of TSH (r = 0.4748, p value = 0.00051 within 95% confidence limit). There was an inverse correlation of duration of HAART with serum FT4 levels. (r = 0.4575, p value = 0.0008, within 95% confidence limit). Also it was seen that the levels of FT3 numerically decreased with the increase in the duration of HAART however, it could not reach statistical significance. (r = 0.2692, p value = 0.0587). The co-efficient of the linear regression also revealed that for every increase in HAART duration by 1 month, the levels of TSH would increase by 0.0304 mIU/L. On the contrary to the current literature, more percentage of males than females had thyroid dysfunction and none had high anti-TPO levels. No correlation was found between thyroid dysfunction and any particular class of drug used in HAART.

The cases in subgroup A had significantly higher TSH and significantly lower FT3/FT4 and anti TPO as compared to subgroup B (p < 0.001). Stepwise linear regression revealed that HIV infection per se along with longer duration of HIV infection, lower CD4 cell counts, exposure to HAART by itself along with longer duration of HAART, male sex, and past history of tuberculosis were strongest predictors of clinical or subclinical thyroid dysfunction in patients with HIV/AIDS. However, multiple regression analysis revealed that infection by HIV per se along with severity of immune suppression (i.e., CD4 cell counts) and treatment with highly active antiretroviral therapy (HAART) were the major predictors of thyroid dysfunction in these HIV-infected individuals.

Discussion

According to literature, overt or subclinical thyroid

dysfunction occurs at almost similar or slightly increased rates in HIV-infected individuals as compared to the general population[5]. Moreover, HAART therapy can complicate thyroid functions further through drug interactions and the immune reconstitution inflammatory syndrome[6]. However, contrary to the existing literature our study found that thyroid dysfunction is rampant in people living with HIV/AIDS and occurs much more frequently in patients receiving HAART. Mean TSH levels were significantly higher in our cases as compared to controls (5.12 vs 3.81 mIu/ml). Even the cases in subgroup B had significantly higher mean TSH and levels FT3/FT4 as compared to controls meaning that HIV seems to be the major culprit behind causation of hypothyroidism (clinical or subclinical) in HIV-infected individuals.

A similar study by Jain et al[7] in 2009 showed higher TSH values(4.135 ± 3.231 mIU/ml) in HIV positive patients as compared to healthy controls. Similarly, higher TSH values were also obtained in HIV-infected individuals by Olivieri et al[8]. The overall mean value of TSH obtained was 4.8 ± 2.7 mIu/ml. This finding was contrary to findings by, Lopresti et al[9] where they could not find any increase in TSH levels as compared to controls. The reason behind higher TSH in HIV-infected individuals in our study as compared to other studies was that the mean duration of disease in our study cases was more than five years as compared to two years in other studies. This may have led to more exposure and hence destruction/injury to thyroid gland by viral particles, inflammatory cells and opportunistic infections and hence the injured thyroid gland could not produce enough thyroid hormone leading to higher TSH (by feedback mechanism). The most prevalent thyroid abnormality in our study was subclinical hypothyroidism, prevalence being 30% amongst cases. It was significantly higher when compared with various other studies being reported as 3.5% by Collazos et al in 2003[10], 6% by Ketsamathi et al in 2006[11], 4% by Madge et al in 2006[12] and 10.6% by Bongiovani et al in 2006[13]. The only study having similar prevalence as our study was by Meena et al[14] from India in 2011 who also reported a 30% prevalence of subclinical hypothyroidism in HIV positive individuals. It was observed in our study that the prevalence of subclinical hypothyroidism amongst cases on anti-retroviral therapy was 50% as compared to only 10% in cases not on any antiretroviral therapy. This higher prevalence in subgroup A suggests a definite role of antiretroviral drugs in the derangement of thyroid functions as proposed by Bongiovani et al[13] and Grappin et al[15]. Apart from some probable toxicity by HAART to thyroid gland, immune reconstitution syndrome (IRS) could have also led to thyroid injury. Amongst commonest IRS entities, thyroiditis is quite common, the manifestations of which are mostly mild and subclinical. Apart from that, other nutritional and metabolic deficiencies like hypoproteinaemia (which are common in Indian HIV-infected individuals) could have added to that. Apart from that we could find that anti-TPO antibody levels (and hence thyroiditis) is not a major cause of hypothyroidism in people with HIV/AIDS (contrary to general population) and HIV-related factors majorly affect thyroid endocrinopathy in these individuals.

Hence, the current study also consolidates the fact that much higher prevalence rate with predominant sub-clinical hypothyroidism is found in Indian HIV positive individuals. The study also revealed strong direct correlation between hypothyroidism (clinical or subclinical) and disease severity (i.e., low CD4 cells count), longer duration of HIV infection along with HAART by itself and longer duration of HAART. The linear regression analysis further strengthened the relationship between the worsening of thyroid function tests culminating into subclinical hypothyroidism and these disease parameters. Hence, there lies a future possibility of early use of thyroid function tests and thyroxine replacement as a tool to manage AIDS along with co-morbidities like metabolic syndrome, central obesity, myopathy, depression, osteoporosis, etc., (where subclinical hypothyroidism has been seen to be associated with these pathologies) in HIV-infected individuals.

Surprisingly, males in our study had higher burden of thyroid abnormalities as compared to females and can be explained by the fact that access to health care and ART is significantly earlier and easier for males than females in India. That could have resulted into earlier initiation and hence longer duration of HAART in males, and hence more of thyroid abnormalities.

Our study recapitulates the significance of regular monitoring of thyroid function tests in HIV-infected individuals and more so when they are on highly active anti-retroviral therapy. However, it is still controversial whether the subclinical hypothyroidism in these individuals should be treated or not, with no current guidelines for the same (at least in people with HIV/AIDS).

Limitations

Our study had majority of HIV-infected individuals in intermediate-to-advanced immune deficiency state with mean CD4 cell counts of 308.16/μl. The higher prevalence of thyroid dysfunction in the study population might be due to the lower CD4 cell counts in subjects. Apart from that, the patients were taken from the ART centre, half of whom were on pre-treatment with combination of three anti-retroviral drugs so the role of single antiretroviral drugs could not be evaluated in the study. Viral load testing was not done and hence the correlation of abnormal TFT with disease severity by measurement of CD4 cell counts

(immunological failure) cannot be extrapolated to virological failure (by viral load testing). Further larger prospective studies with higher number of HIV positive individuals may be the need of the hour to better assess the clinical impact of subclinical hypothyroidism in HIV-infected subjects.

Conclusion

Thyroid disorders (especially subclinical hypothyroidism) is a very common but under-reported entity in patients with HIV/AIDS and HIV virus per se along with ART seems to be the biggest factor affecting thyroid endocrinopathy.

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A Comparison between Ultrasonogram versus Clinical Parameters to Detect Disease Activity in Rheumatoid Arthritis Patients

Tuhin Subhra Sarkar*, Surajit Das**, Umakanta Mahapatra***, Kalimujjaman Molla*, Kripasindhu Gantait ****

Abstract

Objective: Traditionally, disease activity in a rheumatoid arthritis patient is assessed by clinically detected joint tenderness, swelling, CDAI score, SDAI score, DAS28 ESR, DAS28 CRP. USG Power Doppler also detects disease activity by detecting joint synovial thickening, increased vascularity. This study investigated the relationship between traditional methods (CDAI score, SDAI score, DAS28 ESR, DAS28 CRP) and USG Power Doppler to detect disease activity in rheumatoid arthritis patients.

Method: One hundred rheumatoid arthritis patients are recruited consecutively for this study. We assessed disease activity by clinical joint assessment, global assessment, ESR, CRP, X-ray, and USG Power Doppler study.

Result: Active disease was found in 96, 72, 80, 74, 62, 12 per cent of the study subjects according to USG Power Doppler, CDAI score, SDAI score, DAS28 ESR, DAS28 CRP, X-ray respectively. Comparison between USG Power Doppler with CDAI score, SDAI score, DAS28 ESR, DAS28 CRP, X-ray to detect disease activity is found statistically significant $p = 0.0015, 0.0269, 0.002. 0.0001, < 0.0001$ respectively.

Conclusion: USG Power Doppler study is superior to detect disease activity than CDAI score, SDAI score, DAS28 ESR, DAS28 CRP and X-ray.

Key words: Rheumatoid arthritis, USG power Doppler, disease activity scores.

Introduction

Rheumatoid arthritis is a chronic inflammatory disorder of unknown aetiology. Although there are a variety of systemic manifestations, the characteristic feature of established RA is persistent inflammatory synovitis, usually involves the peripheral joint in symmetric distribution[1]. In the ACR/EULAR 2010 criteria set, classification as "definite RA" is based on the confirmed presence of synovitis in at least 1 joint, absence of an alternative diagnosis that better explains the synovitis, and achievement of a total score of 6 or greater (of a possible 10) from the individual scores in 4 domains: number and site of involved joints (score range 0 - 5), serologic abnormality (score range 0 - 3), elevated acute-phase response (score range 0 - 1), and symptom duration (2 levels; range 0 - 1)[2], with the synovitis not better explained by another disease. Classification criteria for RA (score-based algorithm: add score of categories A-D; a score of 6/10 is needed for classification of a patient as having definite RA. RA is the most common inflammatory joint disease that causes premature mortality, disability, and compromised quality of life, two to three times more common in females than males. RA is widely prevalent throughout the world. The prevalence of RA in the world is 0.8%[3] and in India the prevalence of RA is 0.7%[4].

Traditionally, the disease activity has been evaluated by clinical variables, laboratory measures, and radiographic findings[5-6]. However, clinical evaluation of joint pain and swelling have not been sufficiently reliable, and conventional plain radiography depicts indirect signs of cartilage loss and bony erosions. USG Power Doppler has greatly improved musculoskeletal imaging in rheumatology. Several studies have demonstrated that high frequency US has more efficacy to detect joint effusion and synovitis[7-14]. Doppler ultrasound (US) can detect Synovial thickening (Synovitis), increased vascularity within joints, and changes in the periarticular soft tissues, thus can demonstrate the presence of active inflammation. In this study, we tried to find out the correlation of ultrasonographic findings with clinical disease activity and X-ray in RA patients and its implications for a better clinical utilisation of this imaging technique.

Aims and objectives

The aim of this study is to find out the correlation between ultrasonographic and clinical findings in Rheumatoid Arthritis patients reporting to Rheumatology clinic in a tertiary care hospital in West Bengal. The Objective of this study is to investigate the relationship between traditional methods (CDAI score, SDAI score, DAS28 ESR, DAS28 CRP) and USG Power Doppler to detect disease activity in

*Post-Graduate Trainee, ***Assistant Professor, ****Professor, Department of General Medicine, **Assistant Professor, Department of Radiology, Midnapore Medical College and Hospital, Midnapore - 721 101, West Bengal.
Corresponding Author: Dr Kripasindhu Gantait, Post-Graduate Trainee, Department of General Medicine, Midnapore Medical College and Hospital, Midnapore - 721 101, West Bengal. Tel: 9434243054, E-mail: gkripasindhu@yahoo.in.

Rheumatoid arthritis patients.

Specific objective

a. To find out correlation between ultrasonographic findings and CDAI score.

b. To find out correlation between ultrasonographic findings and SDAI score.

c. To find out correlation between ultrasonographic findings and DAS28ESR score.

d. To find out correlation between ultrasonographic findings and DAS28CRP score.

e. To compare the findings between X-ray and ultrasonography of hands.

Methodology

This study has been conducted in the Rheumatology Clinic of a tertiary care hospital in West Bengal. This study was conducted for a period of one year. One hundred consecutive patients were selected for this study who met the ACR/ EULAR 2010 criteria for rheumatoid arthritis and were more than sixteen years of age and willing to participate in this study. We excluded the patients who has septic and traumatic arthritis and history of hand surgery in the last 12 months. The criteria for inclusion of cases from clinical history, biochemical evidence of Rheumatoid arthritis (based on ACR/EULAR2010). Clinical history of rheumatoid arthritis is early morning stiffness lasting more than one hour and easing with activity of affected peripheral joint. The commonly affected joints are symmetrical wrist, proximal interphalangeal, metacarpophalangeal joints. We assessed patients disease activity by tender joint count, swollen joint count, Global assessment of patient health. We performed blood investigations – complete blood count, ESR, CRP, creatinine, SGPT/SGOT, rheumatoid factor, anti-CCP antibody and radiological investigation (X-ray) and USG Power Doppler study. We then assessed disease activity by calculating their CDAI, SDAI, DAS28 ESR, DAS28 CRP score.

Statistical analysis

Data were entered in Microsoft excel spreadsheet and then analysed by SPSS 24.0 and Graphpadprisom version 5. Data has been summarised as mean and standard deviation for numerical variables and percentage for categorical variables. McNemar Test was done to see statistical sensitivity difference the two tests.

Result

We conducted this study in the rheumatology clinic in our hospital. One hundred consecutive patients were selected for this study. The background characteristics of the patients

are depicted in Table I and disease activity status whether active disease or in remission calculated by different method like CDAI score, SDAI score, DAS28 ESR, DAS28 CRP, X-ray and USG Power Doppler study shown in Table II. We found that most of the study population 40% is from 36 - 50 years of age followed by 16 - 35 yrs age 32% and 28% of study population above the age 50 years of age. The mean age of study population is 43 ± 12.7 years and mean duration of disease is 6.8 ± 6 years. The mean ESR and CRP is 42.8 ± 21.9 and 7.7 ± 12.7 respectively. Male is 26% and female is 74 % of study population. Most of the study population (34%) had studied below class X followed by 28% illiterate, 26% Class X, and only 12% were graduates and above.

Table I: Distribution of study population according to basic information (age, sex and educational status, ESR, CRP, disease duration) (N = 100).

Age group	(16 - 35) Yrs	(36 - 50) Yrs	> 50 Yrs	
	32 (32%)	40 (40%)	28(28%)	
Sex	Male		Female	
	26(26%)	74(74%)		
Education	Illiterate	Below class X	Class X	Graduate and above
	28 (28%)	34 (34%)	26 (26%)	12 (12%)
The mean age of study population in years (Mean ± SD):		43 ± 12.7		
The mean duration of disease of study population in years (Mean ± SD):		6.8 ± 6		
The mean ESR of study population in mm/hour (Mean ± SD):		42.8±21.9		
The mean CRP of study population in mg/dl (Mean ± SD):		7.7±12.7		

Table II: Distribution of study population according to disease activity status by various methods. (N = 100).

Remission	Active disease				
	Remission	Mild	Moderate	Severe	Total active
CDAI score	28 (28%)	20 (20%)	40 (40%)	12 (12%)	72 (72%)
SDAI score	20 (20%)	20 (20%)	36 (36%)	24 (24%)	80 (80%)
DAS28 ESR	26 (26%)	10 (10%)	40 (40%)	24 (24%)	74 (74%)
DAS28 CRP	38 (38%)	10 (10%)	44 (44%)	8 (8%)	62 (62%)
X-ray*	88 (88%)	-	-	-	12 (12%)
USG (Synovitis)**	4 (4%)	-	-	-	96 (96%)
USG (Hyperaemia)***	60 (60%)	-	-	-	40 (40%)

*In X-ray we looked for joint erosion which is suggestive of active disease.

**In USG Doppler study we looked for synovial thickening.

***In USG Doppler study we also looked for increased vascularity.

We found that according to CDAI score 28 (28%) had

remission, 20 (20%) had low, 40 (40%) had moderate, 12 (12%) had high disease activity. According to SDAI score 20 (20%) had remission, 20 (20%) had low, 36 (36%) had moderate, 24 (24%) had high disease activity. According to DAS28 ESR score 26 (26%) had remission, 10 (10%) had low, 40 (40%) had moderate, 24 (24%) had high disease activity. According to DAS28CRP score 38 (38%) had remission, 10 (10%) had low, 44 (44%) had moderate, 8 (8%) had high disease activity.

We found that 22 (22%) had non tender - non swollen, 56 (56%) had tender only, 22 (22%) had tender + swollen joint. We found that 12 (12%) had joint erosion, 88 (88%) had no joint erosion on X-ray. 96(96%) had synovial thickening, 4 (4%) had no synovial thickening on USG. 40 (40%) had hyperaemia, 60 (60%) had no hyperaemia on USG.

We found that the comparison between CDAI score and USG Power Doppler study to detect disease activity is statistically significant (P = 0.0015). USG Power Doppler study is superior to detect disease activity than CDAI score. In comparison between SDAI score and USG Power Doppler study to detect disease activity found statistically significant (P = 0.0269). USG Power Doppler study is superior to detect disease activity than SDAI score. In case comparison between DAS28 ESR score and USG Power Doppler study to detect disease activity is statistically significant (P = 0.002). USG is superior to detect disease activity than DAS28 ESR score. In comparison between DAS28 CRP score and USG Power Doppler study to detect disease activity is statistically significant (P = 0.0001). USG is superior to detect disease activity than DAS28 ESR score. Comparison between X-ray and USG to detect disease activity is found statistically significant (P = < 0.0001). Here USG is superior than X-ray to detect disease activity.

Discussion

With the development of high-frequency ultrasound technology, USG Power Doppler study plays an important role to detect disease activity in rheumatoid arthritis patient. USG Power Doppler study detects disease activity by detecting synovial thickening (Synovitis) and increased vascularity (hyperaemia).

In our study, we found that most of the study population (40%) is from 36 - 50 years of age, followed by 16 - 35 yrs age (32%), and 28% of the study population is above the age of 50 years. The mean age of study population is 43 ± 12.7 years and mean duration of disease is 6.8 ± 6 years. The mean ESR and CRP is 42.8 ± 21.9 and 7.7 ± 12.7 respectively. The ratio of male to female is 1:3 approx. Most of the study population (34%) had studied below class X, followed by 28% illiterate, 26% class X and only 12% were graduates and above.

We calculated CDAI score, SDAI score, DAS28 ESR, DAS28 CRP and performed X-ray to look for any joint erosions, and also USG Power Doppler study was done to see for signs of synovitis or increased vascularity. Remission (inactive disease) was found in 28%, 20%, 26%, 38%, 88% and 4% of study population according to CDAI score, SDAI score, DAS28 ESR, DAS28 CRP, X-ray and USG Power Doppler study respectively. Active disease was found in 72%, 80%, 74%, 62%, 12% and 96% of study population according to CDAI score, SDAI score, DAS28 ESR, DAS28 CRP, X-ray and USG Power Doppler study respectively.

We performed statistical analysis to see if sensitivity to detect active disease by different methods is statistically significant or not. We found USG Power Doppler study is superior to detect disease activity than traditional methods like CDAI score (p = 0.0015), SDAI score (p = 0.0269), DAS28 ESR (p = 0.002) and DAS28 CRP (p = 0.0001); all are statistically significant. USG Power Doppler study is also superior to detect disease activity than X-ray (p = < 0.0001) (statistically significant).

Naredo *et al* have found that USG diagnosed more joints with effusion and synovitis than clinical examination and US findings correlated better with CRP and ESR than clinical measures[11]. Hyper-vascularisation and angiogenesis of the synovial membrane are considered to be primary pathogenic mechanisms responsible for joint destruction. Ninety-four (94) consecutive patients who fulfilled the criteria of 1987 American Rheumatic Association for RA were included in this study. Patients who had had traumatic, septic, or microcrystalline arthritis, previous joint surgery, or isotopic synovectomy within the past 12 months (before the study) were excluded from the study.

Mondal *et al* have done a similar type of study in India. They found that Power Doppler ultrasonography of synovium of small joints of hands and feet is a very useful tool in assessing both inflammatory and destructive changes and help the clinician to start the appropriate medication at the earliest stage of the disease[14].

The relationship of MSUS parameters with synovial tissue features is clearly a field open to research, which may add new pathogenic information and help to clarify MSUS usefulness in RA management. Correlation of MSUS was done with physical examination, inflammatory markers and patient-reported outcomes. For many years, rheumatologists have been using the disease activity score of 28 joints (DAS28) and other composite scores as a gold standard for assessment of RA activity; these tools have clearly brought great progress in treatment monitoring. Even though they are the most extensively validated methods for measuring disease activity to date[15], the precise way of objectively defining inflammation is still lacking.

MSUS is more sensitive than physical examination for detection of arthritis according to a number of studies[16-20].

Conclusion

USG Power Doppler study is a very important tool to assess the disease activity in rheumatoid arthritis patients. It is superior in sensitivity to assess and to detect disease activity than traditional method like CDAI score, SDAI score, DAS28 ESR and DAS28 CRP. Synovitis (Synovial thickening) is commonly detected than hyperaemia in ultrasonography. Power Doppler ultrasonography is also more sensitive than X-ray to detect disease activity.

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Comparison of APRI, FIB-4 with Shear Wave Elastography in Assessment of Liver Fibrosis in Untreated Chronic Hepatitis C Patients

Kanchan Kiran Kujur*, Ravinder Garg**, Shaminder Kaur***, Simmi Aggarwal****, Chaitanya Tapasvi*****,
Sumit Pal Singh Chawla******

Abstract

Background: Hepatitis C virus infection is one of the main causes of chronic liver disease worldwide. The high rates of progression of chronic hepatitis C to liver cirrhosis suggests an emergent need of effective tests to diagnose fibrosis in earlier stages. In this study we aim to compare the diagnostic accuracy of 2 non-invasive and easy to apply markers, i.e., APRI and FIB-4 with Shear Wave Elastography (which is taken as the reference) in detecting liver fibrosis in patients with chronic hepatitis C.

Material and methods: This hospital-based descriptive study was conducted on 70 untreated hepatitis C positive patients who came to outdoor or inpatient department in GGS Medical College and Hospital, Faridkot. All the patients underwent complete clinical evaluation and investigations including Shear wave Elastography (reference for liver fibrosis) and then APRI and FIB-4 Scores were calculated on all the patients.

Results: APRI and FIB-4 showed diagnostic accuracies of 84.29%, 81.43% (mild fibrosis), 78.95%, 92.11% (moderate fibrosis) and 73.68%, 73.68% (severe fibrosis), respectively in predicting fibrosis in untreated in chronic hepatitis C patients.

Conclusion: Both APRI and FIB-4 are simple and noninvasive for predicting mild, moderate, and severe fibrosis in untreated chronic hepatitis C patients in comparison to shear wave elastography. In case of moderate fibrosis, FIB-4 has a higher diagnostic accuracy as compared to APRI.

Key words: Hepatitis C virus, chronic hepatitis C APRI, FIB-4, Shear Wave Elastography.

Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide[1]. There are approximately 130 to 150 million carriers of HCV, out of which 55 - 85% develop the chronic form of hepatitis C and 15 - 30% of these patients are at risk of developing cirrhosis within 20 years of their diagnosis[2]. Even India has a great burden of HCV with a population prevalence of 1 - 2.4%[3,4].

The natural course of HCV infection varies. Liver fibrosis and inflammation in chronic hepatitis C are dynamic processes. The progression of chronic hepatitis C (CHC) to liver fibrosis and then to liver cirrhosis correlates with an extensive accumulation of extracellular matrix (ECM), leading to the formation of large amounts of fibrotic tissue that facilitates the occurrence of cirrhosis and subsequently hepatocellular carcinoma. An early and prompt diagnosis and management of liver fibrosis can prevent complications and death[5].

Till date, liver biopsy has been the gold standard for diagnosing and staging fibrosis, but due to its limitations like invasiveness, associated morbidity (e.g., severe pain,

GI bleeding, etc.)[6,7], sampling errors causing misclassification of stage of fibrosis and intra- and inter-observer variability in interpretation of histology, especially at lower stages of fibrosis, biopsy is no longer recommended[8].

The APRI is an indirect biochemical marker of fibrosis, which takes into account the serum level of aspartate aminotransferase (AST) and platelet count for staging liver fibrosis, with good accuracy[10].

The FIB-4 another score for liver fibrosis uses data routinely available in clinical practice, namely AST, ALT, platelet count and patient's age[11,12].

The Shear wave elastography can detect the presence and extent of fibrosis with fairly high accuracy and thus can replace liver biopsy for assessment of fibrosis. However, this diagnostic modality is not uniformly available at all centres imparting treatment to chronic hepatitis C patients. Thus, there arises an urgent need for other easily available, convenient, and non-invasive modalities for effective detection of fibrosis in patients with chronic hepatitis C infection. Hence, this study was undertaken to compare the diagnostic accuracy of 2 non-invasive and easy to apply

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*PG Resident, **Professor and Head, ***Associate Professor, ******Assistant Professor, Department of Medicine, *****Professor, Department of Radiodiagnosis, Guru Gobind Singh Medical College and Hospital, Faridkot - 151 203, Punjab. ****Consultant Radiologist, Garg Hospital and Diagnostic Centre, Main Bazaar, Faridkot - 151 203. Corresponding Author: Dr Simmi Aggarwal, Consultant Radiologist, Garg Hospital and Diagnostic Centre, Main Bazaar, Faridkot - 151 203, Punjab. Tel: 9356912818, E-mail: drsimmigarg@gmail.com.

markers, i.e., APRI and FIB-4 with Shear Wave Elastography (which is taken as the reference) in detecting liver fibrosis in patients with chronic hepatitis C.

Material and methods

This hospital-based observational study was conducted on 70 hepatitis C positive patients who came to outdoor or inpatient departments in GGS Medical College and Hospital, Faridkot. The study was carried-out from February 2018 to February 2019 after the approval from Institutional Ethics Committee, Guru Gobind Singh Medical College, Faridkot. Written informed consent was obtained from the patients.

The sample size was calculated considering the number of newly diagnosed treatment naïve hepatitis patients that present to GGSMCH, Faridkot each year.

Inclusion criteria

1. Patients of both gender above 20 and below 60 years of age who gave informed consent, and who fulfilled the following criteria:-

   HCV RNA positive by quantitative Polymerase Chain Reaction (PCR) assay, (with a limit of detection of > = 15 IU/ml), not treated for hepatitis-C previously, was included in the study.

Exclusion criteria

1. Patients with co-infection with hepatitis B virus or Human Immunodeficiency Virus (HIV) co-infection.

2. Patients with hepatocellular carcinoma (HCC).

3. Patients with significant alcohol abuse (> 80 grams/day).

4. Patients with ALT flare [values five-fold the upper limit of normal (45 U/ml)].

5. Patients with failed or unreliable liver stiffness measurement.

6. Patients with co-infection with more than one HCV genotype.

7. Pregnancy.

8. NAFLD (Non-alcoholic fatty liver disease).

Study tools

1. The APRI[10] and FIB-4[11] are calculated using the following formulae, as originally reported:-

   - APRI = [AST/(ULN)/ platelet count ($*10^9$/L)] x 100.

APRI uses readily available laboratory tests to identify significant hepatic fibrosis and it is one of the simplest

models proposed by Wai *et al*. It is based on the rationale that worsening of fibrosis and increasing portal pressures are associated with reduced production of thrombopoeitin by hepatocytes, increased platelet sequestration within the spleen and reduced clearance of AST[10].

   - FIB-4 = AST (IU/ml) x age (years)/platelet count ($*10^9$/L) x ALT$^{1/2}$ (IU/ml).

FIB-4 was originally developed to predict significant fibrosis and cirrhosis among human immunodeficiency virus (HIV)/HCV co-infected patients in the APRICOT study[11]. Subsequently, it was validated for HCV mono-infected patients[30].

2. Shear Wave Elastography (SWE) (in kPa) is one of the extensively used non-invasive methods for liver stiffness assessment in recent past. It has attracted attention because it is a novel, rapid, objective, and quantitative method for measuring liver stiffness in patients with liver disease, including CHC liver disease[13]. It gauges liver stiffness by measuring the propagation velocity of mechanical shear waves generated in the liver tissue with results expressed in kPa[9].

Methodology

After taking informed consent, a detailed history of each patient was taken. All of them underwent routine investigations and then APRI and FIB-4 scores were calculated for all the patients by single and same observer. On the same day, liver stiffness measurement was done by Shear Wave Elastography (SWE) using ultrasound machine Philips Affiniti 70. For each patient, the right lobe of liver was visualised through the optimal intercostal space with right arm in maximally abducted position while the patient was lying in supine position. Patient was instructed to hold their breath for 3 - 5 seconds for imaging. The visual depth of the system was fixed at 8 centimeters. The ROI (region of interest) was fixed at 1 - 2 centimeters beneath the right liver capsule (Glisson's capsule), away from intrahepatic vessels, bile duct, and gallbladder. The system was adjusted so that the sample volume depth will be 4 centimeter or less. The ultrasound beam is focused at a given location which creates plane shear waves, which propagate over a region of interest (ROI) of tissue. The particular segment of liver was shot 10 - 12 times and the result was considered reliable only when 10 successful shots and a measurement with success rate of > 80% was obtained. The machine automatically calculated the mean elastic modulus (in kPa) within the region of interest.

The proposed cut-offs for LSM values for HCV patients were as follows:-

Severity of fibrosis	METAVIR stages	LSM values (in kPa)
1. Normal	METAVIR F0-F1	3 - 7
2. Mild fibrosis	METAVIR F2	7.1 - 11
3. Moderate fibrosis	METAVIR F3	11.1 - 21
4. Severe fibrosis	METAVIR F4	> 21

Then, diagnostic accuracies of APRI and FIB-4 were compared with Shear wave elastography in assessing liver fibrosis by using appropriate statistical methods in SPSS version 21.0. A p value of < 0.05 was considered statistically significant and the diagnostic value was classified as low (Az = 0.50 - 0.70), moderate (Az = 0.70 - 0.90) and high (Az = 0.90 - 1.0).

Results

Table I: Frequency distribution of gender, occupation and area.

Demographic variables	Frequency	Per cent
Gender		
Male	55	78.57
Female	15	21.43
Total	70	100.0
Occupation		
Housewife	15	21.43
Labourer	10	14.29
Farmer	20	28.57
Driver	4	5.71
Business	13	18.57
Student	4	5.71
Service	4	5.71
Total	70	100.0
Area of residence		
Urban	18	25.71
Rural	52	74.29
Total	70	100.0

Table II: Table for age distribution

Age group	Frequency	Percentage
21 - 30	15	21.43%
31 - 40	19	27.14%
41 - 50	18	25.71%
51 - 60	18	25.71%
Total	70	100.00%

Table III: Frequency distribution of Shear Wave Elastography findings.

	Frequency (n)	Percentage (%)
Normal	32	45.71
Mild fibrosis	13	18.57
Moderate fibrosis	12	17.14
Severe fibrosis	13	18.57
Total	70	100.00

Table IV: Frequency distribution of severity of liver fibrosis.

	Frequency (n)	Percentage (%)
Mild fibrosis	13	34.21
Moderate fibrosis	12	31.58
Severe fibrosis	13	34.21
Total	38	100.00

Table V: Frequency distribution of modes of transmission of hepatitis C.

Risk Factors		Frequency (n)	Per cent (%)
Blood transfusion	No	67	95.71
	Yes	3	4.29
Unsafe injection use	No	54	77.14
	Yes	16	22.86
IVDU	No	56	80.0
	Yes	14	20.0
Unprotected sex	No	65	92.8
	Yes	5	7.14
Surgery	No	53	75.71
	Yes	17	24.29
Dental treatment	No	56	80.0
	Yes	14	20.0
Others	No	54	77.14
	Yes	16	22.86

Table VI: Association between risk factor and liver fibrosis.

		Liver fibrosis		Total	p-value
		No	Yes		
Age distribution	21 - 30	14 (93.33%)	1 (6.67%)	15 (100.00%)	0.0002*
	31 - 40	9 (47.37%)	10 (52.63%)	19 (100.00%)	
	41-50	4 (22.22%)	14 (77.78%)	18 (100.00%)	
	51-60	5 (27.78%)	13 (72.22%)	18 (100.00%)	
Total		32 (45.71%)	38 (54.29%)	70 (100.00%)	
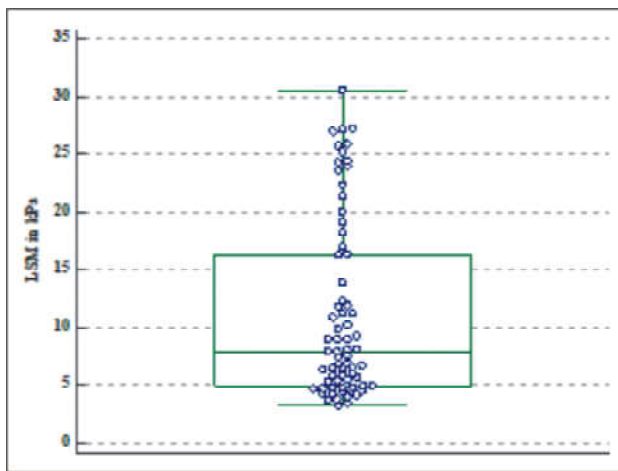
Fig. 1: Box plot for swe LSM values.

Table VII: Descriptive statistics and association between blood investigations parameters and fibrosis.

Blood Investigations	Min	Max	Mean ± SD	Median	Inter quartile range	p-value
Hb (gm/dl)	7.9	16.4	12.9 ± 1.9	12.95	11.6 - 14.3	0.149
TLC (cells/microliter)	3,800	10,600	7,067.14 ± 1,546.92	7,200	5,800 - 8,200	0.662
PLT (/μl)	57,000	4,86,000	2,12,642.9 ± 99,842.9	1,97,500	1,28,000 - 2,95,000	0.001*
AST/SGOT (IU/L)	21	252	95.99 ± 58.95	83	51 - 129	0.0001*
ALT/SGPT (IU/L)	17	224	108.4 ± 65.1	91.5	59 - 164	0.052
ALP (IU/L)	49	187	114.66 ± 30.46	106	92 - 135	0.313
TSP (gm/dl)	5.6	8.0	7.02 ± 0.47	7	6.8 - 7.2	0.204
Albumin (gm/dl)	2.0	4.9	3.64 ± 0.61	3.75	3.1 - 4.1	0.004*
INR	1.0	1.8	1.15 ± 0.17	1.1	1.08 - 1.20	0.046*
HCV viral load (log10 IU/L)	8,305	6,85,00,024	25,93,437.0 ± 85,46,022.5	5,67,000	1,99,000 - 12,77,642	0.203

Table VIII: Association between APRI, FIB-4 and liver fibrosis.

	Liver fibrosis		
	No	Yes	p-value
APRI			<.0001*
Sample size	32	38	
Mean ± SD	0.85 ± 0.77	2.24 ± 1.53	
Median	0.53	1.81	
Min - Max	0.1 - 3.28	0.26 - 8.98	
Inter quartile range	0.364 - 1.137	1.274 - 2.889	
FIB-4			<.0001*
Sample size	32	38	
Mean ± SD	1.26 ± 0.93	3.66 ± 2.4	
Median	0.89	2.96	
Min-Max	0.23 - 3.38	0.93 - 12	
Inter quartile range	0.597 - 1.995	1.790 - 5.090	

Table IX: Association between APRI and FIB-4 with levels of fibrosis.

	Severity of fibrosis			
	Mild	Moderate	Severe	p-value
APRI				0.004*
Sample size	13	12	13	
Mean ± SD	1.3 ± 0.55	2.35 ± 1.07	3.1 ± 2.03	
Median	1.34	2.53	2.5	
Min-Max	0.26 - 2.37	0.92 - 3.9	1.16 - 8.98	
Inter-quartile range	1.111 - 1.576	1.305 - 3.185	2.019 - 3.352	
FIB-4				0.0001*
Sample size	13	12	13	
Mean ± SD	1.82 ± 0.99	3.88 ± 1.71	5.3 ± 2.75	
Median	1.58	3.45	5.09	
Min-Max	0.93 - 4.68	1.37 - 6.97	2.76 - 12	
Inter-quartile range	1.140 - 2.221	2.878 - 4.694	2.871 - 6.504	

Table X: Correlation between variables and liver fibrosis.

	LSM (in kPa)		p-value
Age	Correlation co-efficient	0.451	0.0001*
	n	70	
Albumin	Correlation co-efficient	-0.399	0.0006*
	n	70	
ALP	Correlation co-efficient	0.164	0.1751
	n	70	
ALT/SGPT	Correlation co-efficient	0.14	0.2493
	n	70	
AST/SGOT	Correlation co-efficient	0.479	<0.0001*
	n	70	
BMI	Correlation co-efficient	0.112	0.3579
	n	70	
HGB	Correlation co-efficient	-0.164	0.174
	n	70	
INR	Correlation co-efficient	0.252	0.0355*
	n	70	
PLT	Correlation co-efficient	-0.398	0.0006*
	n	70	
TLC	Correlation co-efficient	0.023	0.8493
	n	70	
TSP	Correlation co-efficient	-0.1	0.41
	n	70	
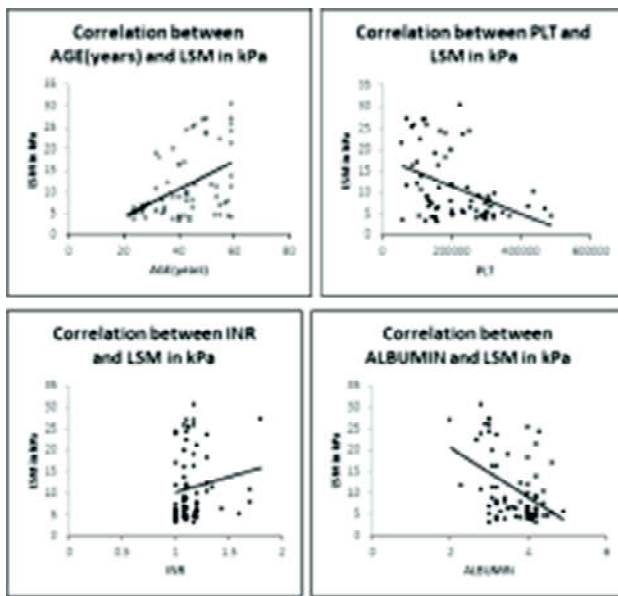
Fig. 2: *Correlation graphs for parameters and liver fibrosis.*

Table XI: Diagnosing mild fibrosis (f2).

Test result variable(s)	Area	Std. error[a]	P-value	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
APRI	0.842	0.05	< 0.0001*	0.735	0.918
FIB-4	0.874	0.0408	< 0.0001*	0.773	0.941

Table XII: Diagnosing moderate fibrosis (≥ f3).

Test result variable(s)	Area	Std. error[a]	p-value	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
APRI	0.818	0.0683	<0.0001*	0.660	0.924
FIB-4	0.926	0.0511	<0.0001*	0.793	0.986

Table XIII: Diagnosing severe fibrosis (f4).

Test result variable(s)	Area	Std. error[a]	p-value	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
APRI	0.735	0.0886	0.0066*	0.567	0.865
FIB-4	0.809	0.07	<0.0001*	0.649	0.918

Table XIV: Comparision of diagnostic accuracies of APRI and FIB-4 for all stages of fibrosis.

	Mild fibrosis	Moderate fibrosis	Severe fibrosis
Difference between areas	0.0321	0.108	0.0738
Standard Error	0.0284	0.0672	0.0639
95% Confidence Interval	-0.0237 to 0.0878	-0.0240 to 0.239	-0.0514 to 0.199
z statistic	1.127	1.602	1.156
p-value	0.2596	0.109	0.248
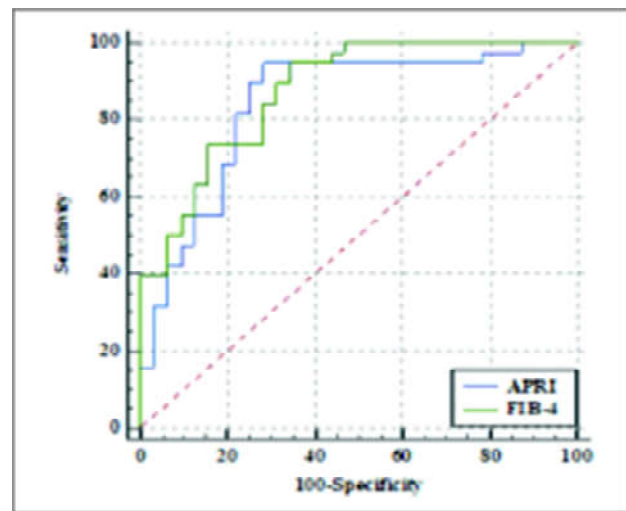
Fig. 3: *ROC curves for comparison of diagnostic accuracies of APRI and FIB-4 for diagnosing mild fibrosis.*
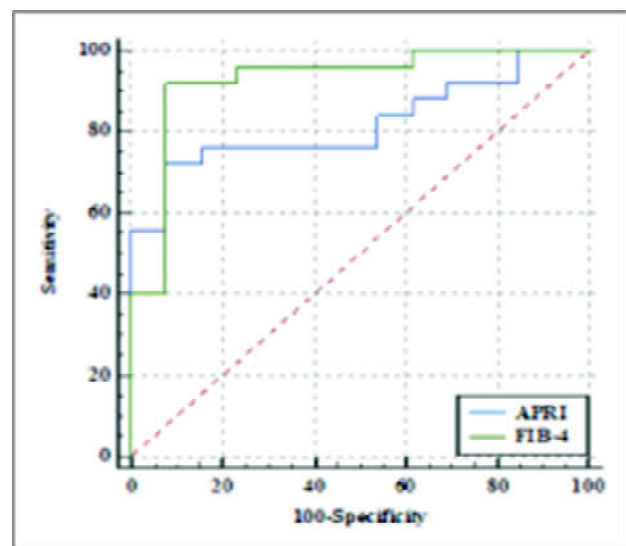
Fig. 4: *ROC curves for comparison of diagnostic accuracies of APRI and FIB-4 for diagnosing moderate fibrosis.*

Discussion

Chronic hepatitis C affects approximately 70 million people worldwide, representing one of the leading causes of liver-related death, hepatocellular carcinoma and liver transplantation. In the present study of 70 newly diagnosed untreated hepatitis C patients, majority belonged to older age groups of 41 - 50 (25.71%), 51 - 60 (25.71%). The study conducted by Jeong *et al* and other studies also had included majority of elderly population[19,20,22].

Most of the subjects included in this study were males (78.6%) and 21% were females similar to other studies (71.9%, 64.3%)[15,22]. In our study, most of the patients resided in rural area (74.3%) while 25.7% resided in urban area.
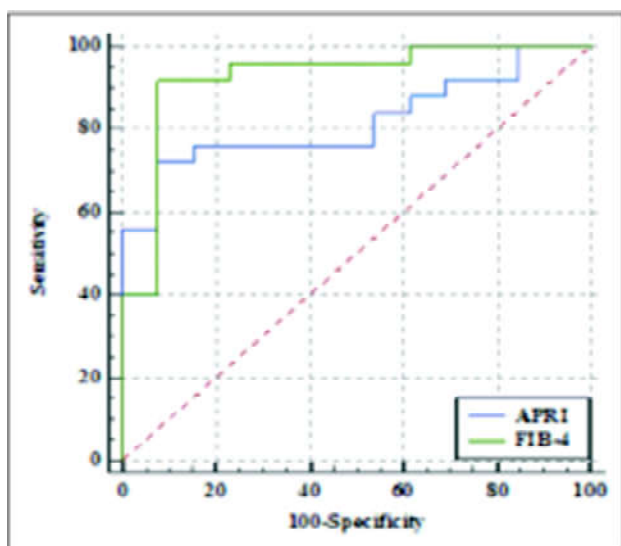
Fig. 5: ROC curves for comparision of diagnostic accuracies of APRI and FIB-4 for diagnosing severe fibrosis.

This could be because of the location of this hospital which caters mostly to rural patients.

In the present study, majority of subjects were farmers (28.6%) followed by housewives (21.4%), owned business (18.6%), labourers (14.3%), drivers (5.7%), students (5.7%) and in-service (5.7%). This could be because the main source of income in this area is agriculture.

The mean BMI in the present study was 25.50 ± 3.47 kg/m^2. According to the WHO and Asia Pacific Obesity classification, most of the patients belonged to overweight class. But, the remarkable feature of SWE is that it can show viscoelastic properties in all areas in the ROI with a colour look-up table. Thus, it overcomes the limitations of Transient Elastography (TE) by which liver stiffness cannot be measured accurately in patients with severe obesity, thick subcutaneous fat or ascites as it was seen in the study by Tada *et al*[18].

In the present study, it was observed that 46% (n = 32) of the patients showed no fibrosis, 19% (n = 13) had mild fibrosis, 17% (n = 12) had moderate fibrosis and only 19% (n = 13) had severe fibrosis.

In this study, we included only the untreated hepatitis C patients because the APRI and FIB-4 values differ as the liver fibrosis reversal occurs with treatment. Tada T *et al* had similar exclusion criteria as seen in our study, except that they excluded chronic hepatitis C (CHC) patients with severe fibrosis (F4)[18].

The present study highlights surgeries to be the most common modes of transmission of hepatitis C (24.3%, n = 17). The other less common modes observed in our study were unsafe injection use (22.9%, n = 16), others (22.9%, n

= 16), IVDU (20%, n = 14), dental treatment (20%, n = 14), unprotected sex (7.1%, n = 5) and blood transfusion being the least common of all (4.3%, n = 3). Others included risk factors like haemodialysis. Unsafe injection use included sharing of needles, tattooing and needle stick injuries. While in the study by Wai *et al*, in 30% patients HCV was transmitted by intravenous drug use, 13% by transfusions of blood or blood products and 26% belonged to others and unknown category[10]. While in other study, the most frequent modes were parenteral drug use (47%), transfusions (22%), and unknown in the remaining patients (31%)[26]. The present study highlights dental treatment, IVDU and unsafe injection use as other important sources of transmission, viz a viz other studies. These are issue of concern and can be attributed to improper sterilisation of surgical and dental equipments. The lack of awareness regarding possible hazards of needle sharing, tattooing and ear piercing from unauthorised professionals may be another contributing factor.

In the present study, all patients who consumed alcohol more than 80 grams per day were not included since, it itself is a major cause of liver fibrosis and all such causes have been excluded. Moreover, the SWE cut-off criteria are different for different aetiologies of chronic liver disease. But, one-third of the total hepatitis C patients in the study (31.4%, n = 22) consumed alcohol occasionally. And, there was no difference among patients with occasional alcohol consumption (i.e., < 30 grams/day) and the non-alcoholics in terms of presence of liver fibrosis (P > 0.05). While, in another study done in PGI Chandigarh, it was reported that there was significantly higher fibrosis in CHC patients even with alcohol consumption of more than 30 grams per day[17].

In the present study, there were statistically significant differences between patients with and without fibrosis when age, platelet count, albumin level and INR were taken into consideration, showing that these were associated with liver fibrosis (i.e., the patients with higher age seemed to have liver fibrosis than patients with younger age. The patients with liver fibrosis had lower platelet counts, albumin levels, and higher INR levels). Also, there was statistically significant difference seen in APRI and FIB-4 scores between patients with and without fibrosis (i.e., the patients with liver fibrosis seemed to have higher levels of APRI and FIB-4 values). Also, we observed statistically significant association between liver fibrosis progression and platelet count, albumin level, APRI and FIB-4. Similar associations were reported in the study by Tada *et al* between platelet level, ALT (p = 0.003, p < 0.001) and grades of fibrosis. But, age was not associated with liver fibrosis unlike in our study. Also, Tamaki and colleagues found similar association when compared age and platelet counts with grades of fibrosis (mild and severe) (p < 0.001, p < 0.001). Both the above two studies compared the serum markers with SWE[18,20]. One of the earliest studies validating the use of APRI and FIB-4 along with another study by Wang

et al had similar associations between patients' age, platelet count, ALT and liver fibrosis, but in these studies liver biopsy was taken as standard[10,12,16].

In this study, significant correlation was observed between patient's development of liver fibrosis in chronic hepatitis C patients and levels of platelet, albumin, and INR. There was statistically significant correlation between fibrosis and platelet count (p = 0.0006) and albumin levels (p = 0.0006) (i.e., the presence of fibrosis, was correlated negatively with platelet count and albumin levels). While, there was a positive correlation between fibrosis and age (p = 0.0001) and INR (p = 0.0355). It seems that growing age plays an important role in the progression of fibrosis. Our observations regarding correlation were similar to various other studies done previously[10,12,22,23,28].

In the systematic review by Parks and co-workers, the various studies assessed by Quality Assessment of Diagnostic Accuracy Studies (QADAS) were taken into consideration. It showed that the predictive values of serum markers and various indices for liver fibrosis assessment were affected by disease prevalence, causing a lack of generalisability to individual practice. Therefore, knowledge of fibrosis prevalence is important to determine aptness of a test in a particular persons' clinical practice. It also inferred that it was possible that some tests performed better in low or high prevalence populations (e.g., test with a high sensitivity across lower test scores, will perform best in low prevalence populations as the NPV will be higher and the test is applicable to a significant part of the study population; and the converse applies in high prevalence populations)[14].

In our study, AUROC of APRI and FIB-4 were 0.842 and 0.874 with the cut-off for APRI and FIB-4 to predict patients with mild fibrosis being > 0.70 and > 1.12 respectively in comparison to SWE taken as reference. Both APRI and FIB-4 scores showed moderate diagnostic accuracy of 84.29% and 81.43% respectively for mild fibrosis. Similar results were reported in earlier study for predicting significant fibrosis, which concluded that APRI and FIB-4 showed moderate diagnostic accuracy similar to the present study, although cirrhosis patients were excluded from one of the studies unlike the present study (0.88); (n = 55, Az = 0.88, 0.86); (0.809, 0.803); (0.77, 0.76); (0.88, 0.85)[10,18,19,21,29]. While, contrasting results were obtained in study by Jeong and co-workers showing APRI (0.691) to have low diagnostic accuracy in comparision to SWE[22].

In the present study, AUROC of APRI and FIB-4 were 0.818 and 0.926 with the cut-off for APRI and FIB-4 to detect moderate fibrosis being > 1.758 and > 2.46 respectively. APRI showed moderate diagnostic accuracy of 78.95% and FIB-4 showed high diagnostic accuracy of 92.11% for predicting moderate fibrosis. Other studies also reported similar results of AUROC and moderate diagnostic accuracy

for APRI and FIB-4 in predicting moderate liver fibrosis (FIB-4 AUROC = 0.85; p < 0.001, < 0.001, Az = 0.819, 0.836; summary AUROC of APRI = 0.77)[12,19,21].

In this study, AUROC of APRI and FIB-4 were 0.735 and 0.809 with the cut-off for APRI and FIB-4 to detect severe fibrosis being > 1.76 and > 2.72 respectively. Both APRI and FIB-4 showed moderate diagnostic accuracy of 73.68% and 73.68% for detecting severe fibrosis. Previous studies reported similar results with AUROCs of 0.815, 0.852 and 0.83, 0.82, respectively indicating moderate diagnostic accuracy for both APRI and FIB-4 tests[19,21]. Holmberg and co-workers in their large US cohort of HCV-infected patients, found that FIB-4 exhibited significantly higher diagnostic accuracy than APRI for differentiating severe fibrosis (stages F3 - F4) from mild-to-moderate fibrosis (stages F0 - F2) (AUROC = 0.83 versus 0.80)[25]. When compared with the earliest studies done using APRI and FIB-4, the present study reported a comparatively lower reliability in diagnosing severe fibrosis (0.94; 0.91)[10,12]. While, other studies reported contrasting results of low diagnostic accuracy of APRI for severe fibrosis (0.683)[22].

Thus, in this study both APRI and FIB-4 diagnosed mild fibrosis from patients with no fibrosis with moderate accuracy and showed least accuracy in differentiating patients of severe fibrosis, while FIB-4 alone showed high reliability in predicting moderate grades of fibrosis in patients with hepatitis C. While several studies concluded opposite results indicating that APRI and FIB-4 exhibit high reliability for predicting liver fibrosis in CHC[12,15,24].

But, the present study finally compared the AUROCs of APRI and FIB-4 in predicting mild, moderate and severe fibrosis and it was observed that both the scores can be used as equally reliable diagnostic tools in predicting various levels of fibrosis in untreated hepatitis C patients. While contrasting findings with statistically significant difference between area under the ROC curves were reported in other studies between SWE and APRI for mild, moderate and severe fibrosis (p = 0.003, 0.032, 0.002)[22].

Strength of the study

The strengths of this study is that we considered necroinflammatory activity grades and did not include the patients with ALT flare (i.e., values higher than five-fold upper limit of normal). In several studies, influence of necroinflammatory activity, jaundice and/or congestion are present in liver[16,27]. Also, very few studies from India have focused on analysis of diagnostic accuracy of simple, non-invasive, inexpensive markers like APRI and FIB-4 for assessment of liver fibrosis in untreated chronic hepatitis C patients. The diagnostic accuracy of these markers was validated in comparison to Shear Wave Elastography which is a valid reference for assessment of liver fibrosis. These

markers can be useful for early detection of liver fibrosis in untreated chronic hepatitis C patients where Shear Wave Elastography is not available or not affordable.

Limitations of the study

The limitations were single-centered study with a small sample size. Further studies with a larger number of patients are warranted.

Conclusion

In conclusion, both APRI and FIB-4 are viable alternatives for predicting mild, moderate, and severe fibrosis in untreated chronic hepatitis C patients in comparison to Shear Wave Elastography. In case of moderate fibrosis, FIB-4 has a higher diagnostic accuracy as compared to APRI.

APRI and FIB-4 can be used as simple, non-invasive tools for assessing liver fibrosis in the majority of chronic hepatitis C patients and can be supplemented with shear wave elastography, where higher degree of fibrosis is suspected.

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ORIGINAL ARTICLE

CSF C-Reactive Protein in Meningitis

Supriya Thakur*, R Loomba**, V Loomba***, M John***

Abstract

Background: Meningitis is defined as an infection involving the subarachnoid space. It may be associated with inflammatory process of the central nervous system (CNS) leading to a decreased level of consciousness, seizures or raised intracranial pressure. CNS is protected by the blood brain barrier (BBB) which resists the entry of pathogens, inflammatory cells, and macromolecules into the subarachnoid space. The BBB may be breached in the presence of infections leading to meningitis. The aetiological diagnosis of meningitis is a diagnostic dilemma. Usually, non specific indicators like CSF glucose, protein concentration, WBC or neutrophil count are used for aetiological diagnosis of meningitis. CSF C-reactive protein (CRP) levels can prove to be a rapid and simple method for specific diagnosis of meningitis. CSF CRP levels in meningitis are raised due to passive diffusion across the inflamed meninges. Normally, CRP is present in trace amounts (< 0.5 mg/dl) in the plasma and in negligible amounts in the CSF.

Material and method: The study was a hospital-based cross-sectional study carried-out from 1st January 2018 to 30th June 2019 in a tertiary care hospital of North India. Total of 110 patients were enrolled for the study. Routine blood and CSF investigations were sent along with CSF CRP and lactate levels.

Results: Mean CSF CRP levels were significantly higher in bacterial meningitis (3.08 ± 1.95 mg/dl) as compared to other types of meningitis (p = 0.005). CSF CRP levels were significantly higher in patients with Thwaites' score > 4 (p = 0.03) and in patients with BM-CASCO score $\geq$3 (p = 0.00001).

Conclusion: CSF CRP levels can help in differrenting bacterial meningitis from other types of meningitis.

Key words: Meningitis, cerebrospinal fluid, C-reactive protein.

Introduction

Meningitis is defined as infection predominantly involving subarachnoid space, associated with CNS inflammatory process leading to decreased or loss of consciousness, seizures, and raised intracranial pressure[1]. Meningitis is a significant cause of morbidity and mortality worldwide. Without adequate treatment the case fatality rate can be as high as 70 per cent, and one in five survivors of meningitis may be left with permanent sequelae including hearing loss or any other neurological disability. Neurological outcome and survival depends largely on damage to central nervous system prior to effective antimicrobial therapy. Quick diagnosis and effective management is the key to success[2]. The aetiological diagnosis of meningitis is a diagnostic dilemma. The cerebrospinal fluid (CSF) biochemical analysis and cellular response overlap in various conditions. In patients with bacterial meningitis, CSF culture and sensitivity is the most reliable method, which is positive in 30 - 60% cases only and requires at least 48 to 72 hours to be positive[3]. Usually non-specific indicators like CSF glucose, chloride, protein level, and WBC or neutrophil count are used for aetiological diagnosis of meningitis. Therefore, CSF C-reactive protein

(CRP) levels can prove to be a rapid and simple method for specific diagnosis of meningitis. C-reactive protein (CRP) is an acute phase reactant of "Pentraxin" group of family[4]. Within 6 hours of an acute inflammatory stimulus, CRP is exclusively produced by hepatocytes and secreted in serum or fluids associated with involved tissues. Tillett *et al* discovered CRP and acute phase inflammatory response in 1930[5]. CSF CRP levels in meningitis are raised due to passive diffusion across inflamed meninges. Normally CRP is present only in trace amount in plasma (< 0.5 mg/dl) and is negligible in CSF. In acute infection, serum CRP concentration can rise up to 3,000 mg/dl within 24 to 48 hours. The concentration of CSF CRP is raised in meningitis[2]. This study is designed to evaluate the diagnostic role of CSF CRP in clinically diagnosed cases of meningitis.

Aims and objectives

1. To study the diagnostic significance of cerebrospinal fluid CRP in evaluation of bacterial meningitis.

2. Correlation of CSF CRP levels with BM – CASCO and Thwaites' scores.

*Senior Resident, ***Professor and Unit Head, Department of Medicine, **Associate Professor, Department of Biochemistry, Christian Medical College and Hospital, Ludhiana - 141 008, Punjab.
Corresponding Author: Dr Rinchu Loomba, Associate Professor, Department of Biochemistry, Christian Medical College and Hospital, Ludhiana - 141 008, Punjab. Tel: 9855601410, E-mail: rinchuloomba@gmail.com.

Material and methods

This study is a hospital-based cross-sectional study conducted in a tertiary care hospital of North India. All patients with clinical diagnosis of meningitis over a period of 18 months were included in the study and subjected to lumbar puncture after informed consent. Routine blood and CSF investigations were sent. CSF was sent for microbiological (cytology, Gram's stain, culture and sensitivity, TB-PCR, Z-N stain) and biochemical (protein, sugar, lactate, ADA, and CRP) analysis. CSF CRP levels were estimated using Beckman Coulter CRP - latex reagent kit on its full automated instrument AU 480.

Sample size: 110 patients.

Inclusion criteria

All patients above the age of 18 years with clinical diagnosis of meningitis were included in the study after informed consent.

Thwaites' and BM-CASCO scores were calculated for all patients and correlated with the CSF CRP levels.

Thwaites' diagnostic scoring[5]

Parameters	Cut-off values	Score
Age (years)	$\geq 35 < 35$	20
Blood WBC (10^3/ml)	$\geq 15,000 < 15,000$	40
History of illness (days)	$\geq 6 < 6$	-50
CSF total WBC (10^3/ml)	$\geq 900 < 900$	30
CSF neutrophils %	$\geq 75 < 75$	40

Bacterial meningitis – careggi score (BM-CASCO)[6]

Blood/CSF parameters	Cut-off calues	Score
CSF cell count (cells/ul)	$\leq 50 > 50$	02
CSF protein (mg/dl)	$\leq 80 > 80$	01
CSF lactate (mg/dl)	$\leq 35 < 35$	01
CSF glucose/serum glucose ratio (%)	$\geq 45 < 45$	01
Peripheral neutrophil count (cells/ul)	$\leq 10,000 \leq 10,000$	01

Statistical analysis

A cross-sectional study was carried-out on 110 patients of meningitis. These patients were divided into the following groups: Bacterial meningitis, Tubercular meningitis, Viral meningitis, Scrub typhus meningitis and Paraneoplastic meningitis based on clinical and CSF findings. Data was entered using Microsoft Office Excel and were analysed by appropriate statistical methods including tests of significance using SPSS (Statistical Packages for Social Sciences, version 21.0. Armonk, NY: IBM corp). Quantitative data was reported as means and standard deviations wherever necessary. Categorical variables were classified as frequency of occurrence and were analysed by using Chi-square test or ANOVA t-test. A p value of < 0.05 was considered significant.

Results and analysis

Total of 110 patients of meningitis were admitted over the period of eighteen months with male preponderance (58.2%). Fever (100%) and headache (80%) were the commonest presenting symptoms (Table I).

Table I:

Symptom	Frequency (n = 110)	Percentage
Fever	110	100
Headache	88	80
Vomiting	55	50
Seizures	43	39.1
Loss of consciousness	14	12.7
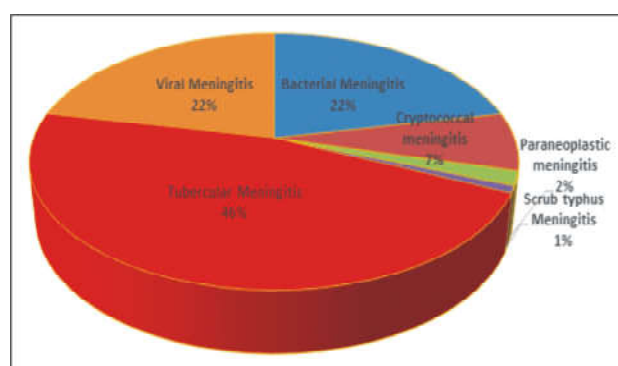
Fig. 1: *Aetiological spectrum of meningitis.*

There were 51 cases of tubercular meningitis, 24 cases of viral and bacterial meningitis each, 8 cases of cryptococcal meningitis, 2 cases of paraneoplastic meningitis and 1 case of Scrub typhus meningitis.
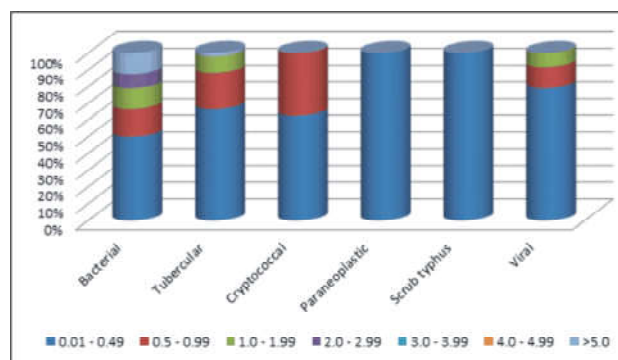
Fig. 2: *CSF CRP in various type of meningitis.*

CSF CRP levels were found to be below 0.5 mg/dl in 73 (66.3%) patients. Only 4 (3.6%) patients were found to have CSF CRP value > 5 mg/dl. Highest levels of CSF CRP (> 5 mg/dl) were found in 3 (12.5%) patients with bacterial meningitis and 1 (1.96%) patient with tubercular meningitis. mean CRP levels were found to be highest in bacterial meningitis (Fig. 3). This result was found statistically significant (p = 0.005).
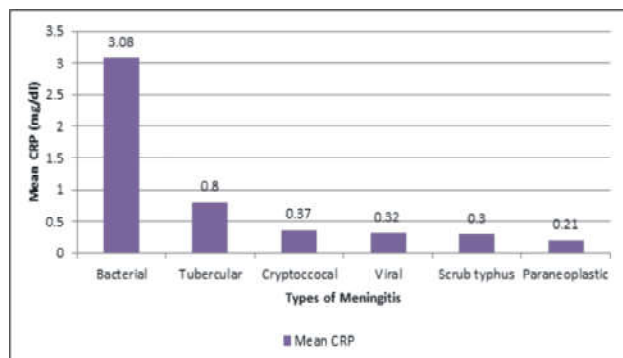
Fig. 1: *Mean CRP.*

48 (43.6%) patients had BM-CASCO ≥ 3 whereas 62 (56.4%) patients had BM-CASCO < 3. When CSF CRP values were correlated with BM-CASCO score, patients having score < 3 were found to have mean CSF CRP value of 0.35 ± 0.31 mg/dl. However, patients with BM-CASCO score ≥ 3 were found to have higher values of mean CSF CRP (2.04 ± 1.12 mg/dl). This result was found to be statistically significant with p value of 0.00001 (Table II).

Table II: Correlation between BM – CASCO scores and mean CSF CRP levels.

BM CASCO score	N = 110	Mean CSF CRP (mg/dl)
< 3	62	0.35 ± 0.31
≥ 3	48	2.04 ± 1.12

87 (79.1%) patients were found to have Thwaites' score ≤ 4. When Thwaites' score was correlated with mean CSF CRP levels, patients having Thwaites' score > 4 were found to have higher mean CSF CRP levels (2.53 ± 2.29 mg/dl) as compared patients having Thwaites' score ≤ 4 with mean CSF CRP level of 0.26 ± 0.19 mg/dl. This is statistically significant with p value of 0.031.

Table III: Correlation between Thwaites' score and mean CSF CRP levels.

Thwaites' score	N = 110	Mean CSF CRP (mg/dl)
≤ 4	87	0.26 ± 0.19
> 4	23	2.53 ± 2.29

Discussion

In our study, 110 patients were included with male preponderance (58.2%). Fever (100%) and headache (86%) were the commonest presenting symptoms and vomiting was seen in 55 (50%) patients, followed by seizures and loss of consciousness in 43 (39.1%) and 14 (12.7%) patients respectively. In this study, 51 cases of tubercular meningitis, 24 cases of viral and bacterial meningitis each, 8 cases of cryptococcal meningitis, 2 cases of paraneoplastic meningitis and 1 case of Scrub typhus meningitis were there.

Highest levels of CSF CRP (> 5 mg/dl) were found in 3 (12.5%) patients with bacterial meningitis and 1 (1.96%) patient with tubercular meningitis. Mean CRP levels were found to be significantly higher in bacterial meningitis (3.08 ± 1.95 mg/dl) as compared to 0.32 ± 0.28 mg/dl in viral meningitis, 0.80 ± 0.52 mg/dl in tubercular meningitis, 0.37 ± 0.36 mg/dl in cryptococcal meningitis, 0.30 mg/dl in Scrub typhus meningitis and 0.21 ± 0.13 mg/dl in paraneoplastic meningitis. This result remained statistically significant with p value = 0.005 (calculated by ANOVA test).

In this study, 87 (79.1%) patients were found to have Thwaites' score ≤ 4. When Thwaites' score was correlated with mean CSF CRP levels, patients having Thwaites' score > 4 were found to have higher mean CSF CRP levels (2.53 ± 2.29 mg/dl) as compared patients having Thwaites' score ≤ 4 with mean CSF CRP level of 0.26 ± 0.19 mg/dl. This difference is statistically significant with p value of 0.031. Hence, Thwaites' score of > 4 can be correlated with higher mean CSF CRP values and bacterial meningitis.

There were 48 (43.6%) patients with BM-CASCO score ≥ 3. When CSF CRP values were correlated with BM-CASCO score, patients having score < 3 were found to have mean CSF CRP value of 0.35 ± 0.31 mg/dl. However, patients with BM-CASCO score ≥ 3 were found to have higher values of mean CSF CRP (2.04 ± 1.12 mg/dl). This result was found to be statistically significant (p value 0.00001). Hence, BM-CASCO score of ≥ 3 can be correlated with higher CSF CRP values and bacterial meningitis.

Prashanth *et al* reported that mean CRP level in CSF of patients was 31.44 mg/dl, 0.86 mg/dl and 1.01 mg/dl in bacterial, tubercular and viral meningitis respectively[7]. Aharwar reported CSF CRP levels above 10 mg/dl in 26 (86.6%) cases of bacterial meningitis. Majority of cases (91.6%) of tubercular meningitis had a CSF CRP range of < 5 mg/dl and one case in range of 10 - 15 mg/dl. Likewise, all cases in viral meningitis group had CSF CRP in the range < 5 mg/dl[8]. Kumar conducted a study at MS Ramaiah Medical College, Bengaluru and mean CSF CRP level was found to be significantly higher in the pyogenic meningitis group

(28.88 mg/l), compared to the non-pyogenic meningitis (1.84 mg/l) and control groups (0.l5 mg/l) which was statistically significant (p < 0.0001)[9]. Similar results were obtained by Belagavi in a study conducted at Tumkur. CRP levels were elevated in pyogenic meningitis. The mean CRP level was 33 ± 5.0 mg/dl. The patients with tubercular and viral meningitis had a mean CRP level of 1.09 ± 0.3 and 1.12 ± 0.48 respectively[10]. Thwaites reported the sensitivity and specificity for prediction of tubercular meningitis as 95.8 and 71.6% respectively, but for patients with positive bacterial result confirmed by microscopy and/or culture evidence of bacteria in CSF the predictive values for sensitivity and specificity were 91.7 and 79.7%, thereby making Thwaites' diagnostic score a useful technique for differential diagnosis of tubercular meningitis. Patients with score ≤ 4 were classified as having tubercular meningitis and with score > 4 were classified as non-tubercular or bacterial meningitis[5]. A study conducted by Lagi during a four-and-a-half year period (2010 - 2014) in tertiary care hospital in Italy reported that all acute bacterial meningitis cases showed a BM-CASCO ≥ 3. Most negative cases exhibited BM-CASCO value of ≤ 1 which was further adopted in the laboratory as a cut-off to not proceed with urgent microbiological analysis of CSF in cases of suspected acute bacterial meningitis in adults. Hence, BM-CASCO score appeared to be an accurate and simple scoring system for optimisation of microbiological diagnosis of acute bacterial meningitis in adults[6].

Conclusion

CSF CRP can be used as biochemical marker of bacterial meningitis. CSF CRP levels can be correlated with BM-CASCO and Thwaites' scores to reach the diagnosis of bacterial meningitis.

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Prognostic Value of CRP in Acute Ischaemic Stroke

Mahesh Dave*, Akhil Vignesh DS**

Abstract

Introduction: Stroke is a worldwide health problem. It makes an important contribution to morbidity, mortality, and disability in developed and developing countries. Stroke is one of the leading causes of death after ischaemic heart disease and malignancy. C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma. Present work was conducted to address the role of CRP in acute stroke patient and its correlation with severity and outcome.

Material and methods: It was hospital based randomised case control observational study in which 50 patients of acute ischaemic stroke admitted in various medical wards of MB Govt Hospital and RNT Medical College, Udaipur were enrolled as cases and 50 healthy age and sex matched individuals were enrolled as controls. The study was carried-out between June 2019 and November 2019.

Results and observations: All these 50 patients of ischaemic stroke confirmed by CT and MRI were classified on the basis of severity according to NIHSS stroke classification in mild (1 - 4), moderate (5 - 15), severe (16 - 20) and very severe (21 - 42) type of stroke. CRP level of all these patients were sent at the time of admission and mean CRP levels were calculated and it was compared with both the groups and correlated with severity and outcome.

Conclusion: From the present study it can be concluded that higher the levels of CRP, the greater will be the severity of stroke and poorer will be the outcome.

Key words: CRP, C-reactive protein, stroke, NIHSS scores.

Introduction

Stroke is a worldwide health problem. It makes an important contribution to morbidity, mortality, and disability in developed as well as developing countries. Stroke is one of the leading causes of death after ischaemic heart disease and malignancy[1].

The World Health Organisation defines stroke as "rapidly developing clinical signs of focal or global disturbance of cerebral function with symptoms lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin"[2] which may be caused either due to interruption of blood supply to the brain or rupture of blood vessels of brain.

According to the WHO, worldwide 15 million people suffer from stroke every year. Out of this, there are around 5 million deaths and another 5 million are permanently disabled. High blood pressure contributes to more than 12.7 million strokes worldwide. In developed countries the incidence of stroke is declining due to efforts made in reducing the blood pressure and curtailing smoking. However, the overall rate of stroke remains high due to aging of the population. Around 70% of strokes and 87% of stroke-related deaths that occur globally are seen in middle and low income countries[3].

Stroke is one of the major killer disease worldwide and also in India, and its prevalance is increasing day by day. In 1998 - 1999, a study conducted in Kolkata showed that the crude prevalence rate of stroke was 147/1,00,000 population and annual incidence rate was 36/1,00,000 population .This was further increased to the level of 262 per 1,00,000 in rural areas and 424 per 1,00,000 in urban areas in the last decade[4].

On comparison of stroke rates in India and other countries, it was found that the rates are two to three times higher in India. The underlying reason for this increase is not yet completely understood. In India, rheumatic heart disease and cortical venous thrombosis are the two most important causes of stroke in the young. This may be partially responsible for increased stroke rates in India[5].

The clinical manifestations of stroke are highly variable because of the complex anatomy of the brain and its vasculature. There are so many factors which can affect the outcome of these patients and it includes age, sex, site, and size of the lesion, presence or absence of midline shift, GCS, TLC counts, temperature, blood glucose, blood pressure at the time of admission.

*Senior Professor and Unit Head, **Resident, Department of Medicine, RNT Medical College, Udaipur - 313 001, Rajasthan.
Corresponding Author: Dr DS Akhil Vignesh, Resident, Room 412, Dilshad Bhawan, PG Boys Hostel, Near Apple Dental Clinic, Chetak Circle, Udaipur - 313 001, Rajasthan. Tel: 9486550050, E-mail: akhilvignesh3@gmail.com.

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma. It is secreted by liver[6] in response to inflammation. It is an acute-phase protein and it increases following interleukin-6 secretion by macrophages and T-cells[7]. It binds to lysophosphatidylcholine expressed on the surface of dead or dying cells or some bacteria so that it can activate the complement system via C1q[8].

It was first discovered by Tillett and Francis[9] in 1930 and was initially thought that it might be secreted by abnormal cells like cancer cells, inflammation, etc. But later it was proved that it is a native protein synthesized and secreted by liver cells[10].

In recent years, inflammation has emerged as an important factor in the genesis of atherosclerosis. There has been a lot of work regarding the role of CRP in cardio-vascular diseases like acute MI and they found a positive correlation of this inflammatory marker with these cardio-vascular disorders whereas there were very few works regarding the role of CRP with acute stroke patients. Hence, the present work was conducted to address the role of CRP in acute stroke patients and its correlation with severity and outcome.

Aims and objectives

1. Estimation of CRP in acute ischaemic stroke and comparison with healthy control patients.

2. To study the correlation between CRP level and severity in acute ischaemic stroke patients.

3. To study correlation between CRP level and short-term outcome at the end of first week.

Material and methods

Study site: Patients admitted in various medical wards of MB Govt Hospital and RNT Medical College, Udaipur.

Study design: Hospital-based randomised case control observational study.

Study period: All eligible 50 consecutive cases of ischaemic stroke admitted in various medical wards of MB Govt Hospital and RNT Medical College, Udaipur were studied from the period June 2019 to November 2019.

Study population: All the patients who presented within 72 hours of onset of symptoms and signs of acute ischaemic stroke and confirmed radiologically with CT or MRI and fulfilling inclusion and exclusion criterias were enrolled. Baseline data were collected within 24 hrs of admission.

Inclusion criteria

1. Acute ischaemic stroke patients of both the sexes having age more than 18 years were considered as cases.

2. 50 healthy age and sex matched individuals who were not suffering from any inflammatory, infectious, auto-immune, or malignant diseases were considered as controls.

Exclusion criteria

1. Patients who are having age less than 18 years.

2. Patients who presented with symptoms suggestive of stroke but with underlying aetiology, e.g., trauma, surgery, neoplasm, active infections.

3. Patients with stroke associated with haematological diseases, inflammatory diseases, severe hepatic illness, renal diseases, acute metabolic diseases, autoimmune diseases, intoxications with cocaine and amphetamines and acute coronary syndromes.

Study method

All these 50 patients with history of acute stroke admitted to the various medical wards of MB Govt Hospital and RNT Medical College, Udaipur were enrolled for the study after taking written consent. Detailed clinical profile of the patients were recorded on separate proforma regarding general information, i.e., age, sex, present and past history, history of systemic illness, and a thorough clinical examination including detailed neurological examination was done. All cases were evaluated clinically including diabetes mellitus, hypertension, ischaemic heart disease, previous history of stroke, alcohol intake, and smoking. All routine haematological investigations including haemoglobin, total and differential leucocyte counts, peripheral blood film examination, urine examination, blood sugar, urea, lipid profile, electrocardiogram, X-ray chest, and computerised tomogram and/or MRI were performed.

All these 50 patients of ischaemic stroke confirmed by CT and MRI were enrolled as cases and then they were classified on the basis of severity according to NIHSS stroke classification in mild (1 - 4), moderate (5 - 15), severe (16 - 20) and very severe (21 - 42) type of stroke.

The CRP levels of both these cases and controls were estimated. The CRP levels of these cases were sent at the time of their admission.

All these enrolled patients were followed-up and outcome was observed and interpreted at the end of 7 days in the form of death, deterioration, no change, improved, and complete recovery.

Mean level of CRP in the cases were compared with the controls and its correlation with the severity and outcome

of stroke was studied.

Statistical analysis

Summary statistics were done by proportions, mean, median, and standard deviation. The inferential statistics were done by, ANOVA and person correlation. All measurements were done using SPSS version 21.0. 'p' value < 0.05 were considered as statistically significant.

Observations and results

Table I: Age-wise distribution.

Age (years)	No. of cases (n = 50)
18 - 40	4 (8%)
41 - 60	14 (28%)
> 60	32 (64%)

Out of 50 cases, 32 (64%) belonged to > 60 years of age followed by 41 - 60 (28%) and 18 - 40 (8%) years age group.

Table II: Gender-wise distribution.

Gender	No. of cases (n = 50)
Male	33 (66%)
Female	17 (34%)

In the present study, 33 (66%) were males and 17 (34%) were females.

Table III: Comparison between NIHSS score CRP.

NIHSS Score	1 - 4	5 - 15	16 - 20	21 - 42
No. of cases (50)	0	17	0	33
Increased CRP (38)	0	6 (6.68 ± 2.60)	0	32 (7.76 ± 2.82)

The above table shows the comparison between CRP levels in acute ischaemic stroke and the severity of stroke as assessed by NIHS scores.

Table IV: Comparison CRP levels between cases and controls.

Age group	Mean CRP levels controls	Mean CRP levels cases	P value
18 - 40	2.85 ± 1.72	3.4 ± 1.45	0.00128
41 - 60	2.66 ± 1.66	5.77 ± 2.86	
> 60	–	6.87 ± 3.54	

The CRP levels of cases and controls were studied. The above observations were made which showed that the levels of CRP were elevated significantly in cases as compared to that of controls.

Table V: Outcome in relation to CRP.

CRP	No. of Cases	Deaths	Deteriorated	No change	Improved	Complete recovery
0 - 3 Mean CRP: 2.16	12 (24%)	1 (8.3%)	0	0	7 (58.33%)	4 (33.33%)
> 3 Mean CRP: 7.72	38 (76%)	17 (44.7%)	11 (28.9%)	4 (10.52%)	6 (15.78%)	0

The outcome was divided into death, deteriorated, no change, improved, and recovery. The outcome was noted within 7 days of admission in the hospital. The cut-off for normal CRP was 3 mg/l above which all the observations made were considered as abnormal.

Discussion

Acute ischaemic stroke is a major cause of disability and deaths in our country. With the advent of promising therapies for acute ischaemic stroke, there is higher expectation for rapid recovery and good outcome. Inspite of these new therapies, prognosis is still not very good which may be because of the fact that its outcome may be influenced by many factors like extent of brain injury, severity and duration of insult, as well genetic predisposition, temperature, blood glucose, and various other unknown factors. Globally, stroke is the third commonest cause of moratlity[11] and the fourth leading cause of disease burden[12].

C-reactive protein is an acute phase reactant which increases during any acute inflammation. Increased CRP levels have also been described in smokers, sedentary and obese persons, malignancy, type 2 diabetes, gestational diabetes, and metabolic syndrome persons[13].

In the present study, 50 patients were included who suffered from acute ischaemic stroke and confirmed by clinical and radiological examinations. The concentrations of CRP were measured in all patients.

In our study, 64% (32 patients) of the patients belonged to the age group of above 60 yrs with 28% (14 patients) belonging to the age group between 41 to 60 yrs. Only 8% (4 patients) of the total population belonged to less than 40 yrs. This indicates that the most common age of presentation of stroke is old age that is above the age of 60. Hayes *et al*[14] 2011 found that the risk of stroke doubles with each decade after 45 yrs and over 70% of all strokes occur above the age of 65. Similar findings were noted by Kissela *et al* 2012 and Roy-O'Reilly *et al* 2018.

Regarding the influence of sex, we found that 66% (33 patients) of the total cases were male and 34% (17 patients)

of the total cases belonged to the female community. Among the females affected, all of them belonged to post-menopausal age. Thus maximum number of affected patients were males. This finding is inaccordance with the study conducted by Haast et al[15] who found that pre-menopausal women are protected from stroke and other cardiovascular events due to the presence of oestrogen. However, after menopause they are affected by stroke at a similar incidence as compared to the male community. Wang et al 2019 also noted the similar findings.

In our study, the severity of stroke was classified according to NIHSS stroke classification in mild (1 - 4), moderate (5 - 15), severe (16 - 20) and very severe (21 - 42) type of stroke. Among the 17 patients with score between 5 - 15 (moderate stroke) 6 patients had raised CRP. The mean CRP was 6.68 ± 2.60 in this group. In the 33 patients with score between 21 - 42 (very severe stroke), 32 patients had raised CRP. The mean CRP was 7.76 ± 2.82. The p value was calculated between both groups. It was 0.00014 when the mean CRP levels of NIHS score in the 21 - 42 group was compared with that of group with scores 5 - 15. Thus we can reasonably conclude that higher levels of CRP are seen in patients with poorer NIHSS scores. The findings published by Geng et al in 2016 also show the same that higher the levels of CRP, the greater will be the NIHSS scores and poorer will be the outcome.

The CRP levels of controls and cases were studied. In the age group of 18 - 40, the mean CRP level in controls was 2.85 ± 1.72, while in cases it was 3.4 ± 1.45. In the age group of 41 - 60, the mean CRP was 2.66 ± 1.66 while that in cases it was 5.77 ± 2.86. Above the age group of 60, the CRP levels in controls were zero as no subjects in this age group were studied while that in cases was 6.87 ± 3.54. The p values were calculated in each group. It was found to be 0.00012 for CRP levels of cases as compared to controls. This is consistent with the findings published by Napoli et al 2001 and Yu et al 2014.

Regarding the correlation with CRP with outcome of the stroke, the following observations were made. The outcome was divided into death, deteriorated, no change, improved, and recovery. The outcome was noted within 7 days of admission in the hospital. The cut-off for normal CRP was 3 mg/l above which all the observations made were considered as abnormal. Of the total number of patients with normal CRP (24%), i.e., 0 - 3 mg/l there was 1 (8.3%) death. 7 (58.33%) patients improved and 4 (33.33%) patients underwent complete recovery. The number of patients with raised CRP (more than 3 mg/l) was 38 (76%) cases. Of these 38 patients, there were 17 (44.7%) deaths, 11 (28.9%) patients condition deteriorated and 4 (10.52%) patients had no significant improvement in their condition. An improvement in condition was noted only in 6 (15.78%)

patients while complete recovery was not seen in any. The poor outcome was considered as those cases that died or deteriorated. The mean CRP levels were 2.16 in the group with normal CRP and 7.72.43 in the group with raised CRP. The p value was calculated between both groups. The p value was 0.0002. The result is significant at p value < 0.05.

In the study conducted by Hertog et al[16] in 2009 it was found that acute ischaemic stroke may give rise to an inflammatory response that leads to raised levels of C-reactive protein (CRP). High levels of CRP may be associated with poor outcome. The study evaluated the prognostic value of CRP within 12 h of onset of ischaemic stroke. Levels of CRP were routinely measured within 12 h of symptom onset in 561 patients with acute ischaemic stroke. CRP values were divided as < 7 or $\geq$ 7 mg/l. They studied the relation between CRP values and poor outcome (modified Rankin scale score > 2) or death at 3 months. A multiple logistic regression model was applied to adjust for age, sex, NIHSS score, current cigarette smoking, diabetes mellitus, hypertension, statin use, and stroke subtype. It was found that patients with CRP levels $\geq$ 7 mg/l had a significantly increased risk of poor outcome or death at 3 months. In addition, the risk of poor outcome or death at 3 months increased with higher levels of CRP. CRP within 12 h of ischaemic stroke is an independent prognostic factor of poor outcome at 3 months.

Conclusion

Stroke is one of the most common and devastating disorders and is the second leading cause of death worldwide. Incidences of stroke are increasing day by day in both developed and developing countries including India.

The clinical manifestation of stroke is highly variable. There are a lot of factors which can affect the outcome in these patients, e.g., TLC levels, extent of lesion, blood sugar levels, etc. Although the role of all these factors have been extensively investigated, very few studies have been published so far regarding the role of CRP in stroke.
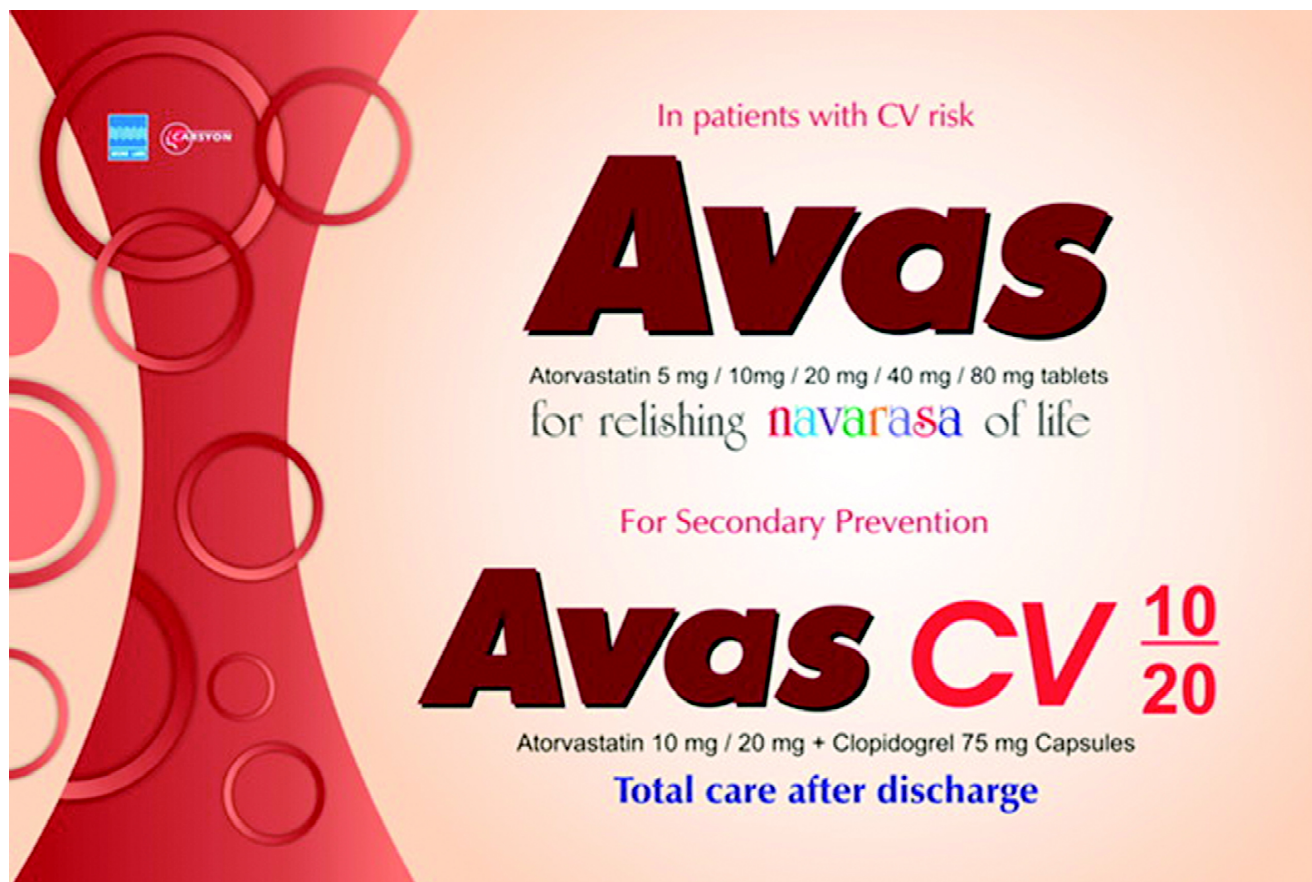
From the present study we can conclude that the CRP levels are usually significantly high in cases of acute ischaemic stroke as compared to that of normal healthy population and the levels of CRP at the time of admission of stroke will predict the outcome and severity of stroke.

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Comparison of RDW with APACHE II in Patients of Severe Sepsis for Prognostication

Aniket Goenka*, CC Chaubal**

Abstract

Sepsis and severe sepsis are a common cause of admission of a patient in intensive care unit and are commonly associated with high mortality rates. RDW is calculated by blood analysers and represents the range of variation in the size of red blood cells in blood. APACHE II (Acute Physiology, Age, Chronic Health Evaluation II) score is a commonly used prognostic indicator which involves 12 variables – both clinical and laboratory. Our study is a single-centre prospective observational study. This study is conducted at People's Hospital of Peoples College of Medical Sciences and Research Centre. Data collection was done from December 2017 to August 2019. All patients received standard medical therapy as per their clinical condition. These patients were then followed-up after 30 days telephonically to check for mortality. The inclusion criterion was severe sepsis patients admitted in ICU or casualty (as defined by the SIRS criteria). Among 32 (54.23%) patients with a score of RDW + APACHEII OF > 29.5, 19 (32.20%) patients did not survive and of the rest 27 (45.76%) with a score of < 29.5, 7 (11.86%) did not survive. From the p value of < 0.001, it can be concluded that there is significant association of higher RDW + APACHE II with mortality.

Introduction

Sepsis, as defined in 2016 by the Sepsis Definitions Task Force, is a life-threatening organ dysfunction caused by a dysregulated host response to infection[5].

Sepsis was earlier defined in the 1991 Consensus Conference, and which largely remained unchanged for about 2 decades by the Severe Inflammatory Response Syndrome (SIRS) criteria[6,7]:-

SIRS is considered to be present when patients have more than one of the following clinical findings:

– Body temperature higher than 38° C or lower than 36° C.

– Heart rate higher than 90/min.

– Hyperventilation evidenced by respiratory rate higher than 20/min or PaCO2 lower than 32 mmHg.

– White blood cell count higher than 12,000 cells/µl or lower than 4,000/µl[6].

Sepsis and severe sepsis are a common cause of admission of a patient to an intensive care unit, and are commonly associated with high mortality rates.

Red cell distribution width (RDW) is a measurement derived from the RBC distribution curves generated on the automated haematology analyser, and is an indicator of variation in RBC size within a blood sample (anisocytosis). RDW is a very commonly available and inexpensive measurement which is a part of all Complete Blood Picture reports.

APACHE II uses a point score based upon initial values of 12 routine physiologic measurements, age, and previous health status to provide a general measure of severity of disease. An increasing score (range 0 to 71) was closely correlated with the subsequent risk of hospital death for 5,815 intensive care admissions from 13 hospitals in a study by Knaus *et al*[2].

Material and methods

Our study is a single-centre prospective observational study. This study was conducted at People's Hospital of Peoples College of Medical Sciences and Research Centre.

Of all the patients who got admitted during the study period in the People's Hospital ICU or casualty, and diagnosed as sepsis clinically, and on the basis of initial investigations as per the Severe Inflammatory Response Syndrome criteria (when patients have more than one of the following clinical findings: body temperature higher than 38° C or lower than 36° C, heart rate higher than 90/min, hyperventilation evidenced by respiratory rate higher than 20/min or PaCO2 lower than 32 mmHg, white blood cell count higher than 12,000 cells/µl or lower than 4,000/µl with a suspicion of infection) were considered as potential participants of the study. On admission of patients suspected to have sepsis, their demographic profile and vitals were noted. The investigations needed for the APACHE 2 score calculations were sent as these investigations anyway are part of baseline investigations needed in a patient of sepsis. The patients identified as *severe sepsis* on the basis of reports

*Post-Graduate Student, **Head, Department of Medicine, Peoples College of Paramedical Science and Research Centre, Karond Bypass Road, Peoples Campus, Bhanpur, Bhopal - 462 037, (MP).
Corresponding Author: Dr Aniket Goenka, Post-Graduate Student, Department of Medicine, Peoples College of Paramedical Science and Research Centre, Karond Bypass Road, Peoples Campus, Bhanpur, Bhopal - 462 037, (MP). Tel: 9340632386, E-mail: ketugoenka@gmail.com.

and clinical data which suggests sepsis with one or more organ failure[7] and after obtaining consent either from the patient or relative of the patient are included in our study.

Inclusion criteria

Severe sepsis patients admitted in ICU or casualty (as defined by the SIRS criteria).

Exclusion criteria

1. Pregnant females.
2. History of blood transfusion in the last one week.
3. Known haematological disorders (leukaemia, myelodysplastic syndrome, neoplastic metastsis to bone marrow).
4. Recent chemotherapy, or immunosuppression, or solid organ transplants.
5. Post-splenectomy.
6. Drugs known to influence morphology of red blood cells.
7. The patients who could not have been reached via telephone call and could not be contacted after one month period were not included in the study.

Results

Table I: Distribution of survivors and non-survivors among the 3 RDW groups.

	Prognosis of subjects		Total
	Non-survivors	survivors	
RDW - CV			
< 14.5 (group 1)	10	17	27
14.6 - 17.3 (group 2)	12	12	24
> 17.4 (group 3)	4	4	8
Total	26	33	59

Chi square value is 0.9 p value 0.6.

Table II: Association of APACHEII + RDW value with mortality.

Parameters	Result	N	Mean	Std deviation	P value
RDW CV	Survivors	33	14.712	1.8530	
	Non-survivors	26	14.962	2.2239	0.64
APACHE II	Survivors	33	13.152	4.1164	

RDW + APACHEII	Non-survivors	Survivors	Marginal row table
< 29.5	7	20	27
> 29.5	19	13	32
Marginal row column	26	33	59

Chi square statistic is 6.6473. The p value 0.001.

Table III: Comparison of RDW-CV and APACHEII among survivors and non-survivors.

Parameters	RESULT	N	Mean	Std. deviation	P value
RDW CV	Survivors	33	14.712	1.8530	
	Non-Survivors	26	14.962	2.2239	0.64
APACHE II	Survivors	33	13.152	4.1164	
	Non-survivors	26	20.154	7.6089	0.001

The total number of subjects who were identified as severe sepsis and who participated in our study were 62, of which 3 subjects were lost to follow-up. There are 59 patients who were identified as severely septic and in whom all the data were available. The data entry was done in MS excel sheet. Data analysis was done in SPSS version 24 (IBM INC. New York, USA).

We calculated the mean and standard deviation for continuous data. We divided the groups in RDW as:

a) < 14.5 (Group 1)

b) 14.5 - 17.3 (Group 2)

c) > 17.3 (Group 3)

(as per a previous study by Jandial published in the *Journal of Critical Care*).

The continuous and discrete data were analysed between these groups. Student T test was used for analysing normally distributed continuous variables. Chi-square test was used for discrete data.

RDW values were added to APACHE II values and the patients segregated as above or below 29.5. The figure of 29.5 was arrived on the basis of addition of the upper normal limit of RDW (14.5) and a baseline value of 15 for APACHE II, which has been considered in various studies involving APACHE II to be a threshold of significance to study poor outcomes.

Aamong 32 (54.23%) patients with a score of RDW + APACHE II of > 29.5, 19 (32.20%) patients did not survive and of the rest 27 (45.76%) with a score of < 29.5, 7 (11.86%) did not survive. From the p value of < 0.001, it can be concluded that there is significant association of higher RDW + APACHE II with mortality.

Table I shows that of the 26 (44.06%) non survivors, 10 (16.94%) are in group 1, 12 (20.33%) in group 2, and 4 (6.77%) in group 3. While among the 33 (55.93%) survivors, 17 (28.81%) are in group 1, 12 (20.33%) in group 2, and 4 (6.77%) in group 3.

Table III shows that there were 33 (55.9%) survivors and 26 (44.1%) non-survivors. APACHE II in survivors had a mean value of 13.152 with a standard deviation of 4.11; and among non survivors with a mean value of 20.154, with a standard

deviation of 7.60, with a p value of < 0.001 was found to be a significant independent predictor of mortality. RDW in survivors had a mean value of 14.712 with a standard deviation of 1.85 and among non survivors a mean value of 14.962 with a standard deviation of 2.23 showed no independent significance in estimating one-month mortality.

Conclusion

The study confirms the value of APACHE II as a predictor of mortality and also identifies the value of RDW on addition to APACHE II as a predictor of the same. The study also identifies that RDW fails to independently prognosticate regarding mortality in patients of severe sepsis.

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A Study of the Cardiac Manifestations in Rheumatoid Arthritis Patients with Special Reference to Echocardiography

Sadanand Khatnawliya*, Archana Gokhroo**, Ramit Singh Pala***, Puneet Patel***

Abstract

Introduction: Cardiac abnormalities have been documented in several studies using echocardiography and Doppler analysis in various European and western countries. Studies have shown pericardial effusion, valvular abnormalities, pulmonary hypertension and cardiac conduction defects, and ventricular dysfunctions. The present study is therefore undertaken to evaluate the various cardiac manifestations including echo-Doppler evaluation of left ventricular function in Indian patients suffering from rheumatoid arthritis.

Material and methods: The study was performed on patients attending the out-patient and in-patients departments of medicine at JLN MC and Hospital, Ajmer. A total of 60 rheumatoid arthritis patients were enrolled. Patients satisfying the revised ACR-EULAR classification criteria (2010) for RA were included in the study. Any type of heart diseases or any other disease affecting heart directly or indirectly were excluded from the study. Total duration of the study was 2 years.

Results: The most common cardiac abnormality seen in this study was left ventricular diastolic dysfunction in 21.6%. Other abnormalities included pericardial disease in 10%, pulmonary hypertension in 6.66%, mitral regurgitation in 5%, and tricuspid regurgitation in 1.66%.

Conclusion: Cardiac abnormalities are largely sub-clinical, hence the early detection of cardiac abnormalities especially by echocardiography can be very important in the correct assessment and management of the RA patients.

Key word: Rheumatoid arthritis, left ventricular diastolic dysfunction, pericardial disease.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown aetiology marked by a symmetric peripheral polyarthritis. It is the most common form of chronic inflammatory arthritis and often results in joint damage and physical disability. Because it is a systemic disease, RA may result in a variety of extra-articular manifestations including fatigue, subcutaneous nodules, lung involvement, pericarditis, peripheral neuropathy, vasculitis and haematologic abnormalities[1].

It has a progressive course with exacerbations and remissions being part of its natural history. Its onset could be at any age, although it usually starts in the fourth decade of life. Overall, there is a 3:1 female preponderance, but this excess is greater in young people and the age-related incidence is approximately equal in elderly people[2].

The incidence of RA increases between 25 and 55 years of age, after which it plateaus until the age of 75 and then decreases[3]. The presenting symptoms of RA typically result from inflammation of the joints, tendons, and bursae. Patients often complain of early morning joint stiffness lasting more than 1 h that eases with physical activity. The earliest involved joints are typically the small joints of the hands and feet. The initial pattern of joint involvement may be monoarticular, oligoarticular (< 5 joints), or polyarticular (> 5 joints), usually in a symmetric distribution[3].

Though being principally a disease of joints, several extra-articular manifestations are also noted. The systemic manifestations include involvement of cardiac, pulmonary, haematological, ocular, and neurological systems. The prevalence of rheumatoid arthritis is between 0.7% to 1.5%.

Many cardiac lesions have been described since then including pericardial effusion, constrictive pericarditis, mitral regurgitation, mitral stenosis, aortic root dilatation and aortic regurgitation, left ventricular systolic and diastolic dysfunction, pulmonary arterial hypertension in many Western and European studies.

Pericardial disease is a common autopsy finding in patients with rheumatoid arthritis, but is frequently asymptomatic during life.

*Senior Resident, Department of Medicine, AIIMS, Ansari Nagar, Delhi - 110 029; **Senior Professor, ***Junior Resident, Department of Medicine, RNT Medical College, Udaipur - 313 001, Rajasthan.
Corresponding Author: Dr Archana Gokhroo, Department of Medicine, RNT Medical College, Udaipur - 313 001, Rajasthan. Tel: 7014273353, E-mail: archanagokhroo@gmail.com.

Material and methods

The study was performed on patients attending the out-patient and in-patient departments of Medicine, JLN Medical College and Hospital, Ajmer. A total of 60 rheumatoid arthritis patients were enrolled. All cases of rheumatoid arthritis of both sexes from eighteen years, who fulfill inclusion and exclusion criteria were studied for a period of two years – from August, 2015 to July, 2017.

Inclusion criteria

- Patients aged above 18 years.

- Patients who were already diagnosed to have RA, come to OPD for non specific complaints (who were satisfying New ACR-EULAR Criteria).

- Patients who were satisfying New ACR-EULAR (American College of Rheumatology and the European League Against Rheumatism 2010) classification criteria.

Exclusion criteria

- Congenital heart disease.

- Ischemic heart disease.

- Valvular heart disease with rheumatic history (Rheumatic heart disease).

- Diabetes mellitus.

- Chronic obstructive pulmonary disease.

- Essential hypertension of more than one year duration.

- Severe anaemia (Hb < 6 gm%).

- Thyroid dysfunction.

- Drug-induced cardiac abnormalities, i.e., beta-blockers, lithium, oral contraceptives, anti-arrhythmic agents, etc.

- Any other disease affecting heart directly or indirectly.

A detailed history – age, sex, duration of RA, presence and duration of morning stiffness, chest symptoms, list of painful joints, presence of other systemic disease, and history of extra-articular manifestations of RA were documented. Treatment history was also documented. Functional status of the patients was recorded on the Steinbrocker's scale. A systemic examination of all joints was done for features of activity, tender joint count and swollen joint count estimation was done. A simplified 28 joint articular index as described by Fuch's *et al* was used to assess disease activity. Twenty-eight joints included 10 proximal interphalangeal joints of the fingers, 10 metacarpophalangeal joints, and the wrist, elbow, shoulder and the knee joints bilaterally[4].

Cardiovascular examination was done in detail. Abdominal, respiratory and neurological examination was also done. Extra-articular manifestations were carefully looked for and documented.

This study highlights the cardiac manifestations in RA as they are some of the most profound and reproducible clinical findings. It assesses the CVS parameters in RA patients without other underlying disorder by echocardiography and ECG. Hence, this study aims at studying the cardiac manifestations of RA thereby reassessing the need for early recognition and aggressive management in the form of anti RA drugs therapy aiming at preventing the before-mentioned complications.

To see significant differences between the groups for continuous variables students "t" test (unpaired two tailed) was performed and to see the difference between the means in skewed distribution, Mann Whitney U test was used. For comparing categorical variables Chi square and Fisher's exact test were used, $p \leq 0.05$ has been considered as statistically significant.

Results

The study was done in Department of Medicine, JLN Medical College, Groups of Hospitals, Ajmer between August 2015 to July 2017. Sixty patients of rheumatoid arthritis diagnosed by revised ACR-EULAR criteria (in 2010, a collaborative effort between The American College of Rheumatology (ACR) and The European League Against Rheumatism (EULAR) had fulfilled the inclusion and exclusion criteria. The study group included 42 females (70%) and 18 males (30%).
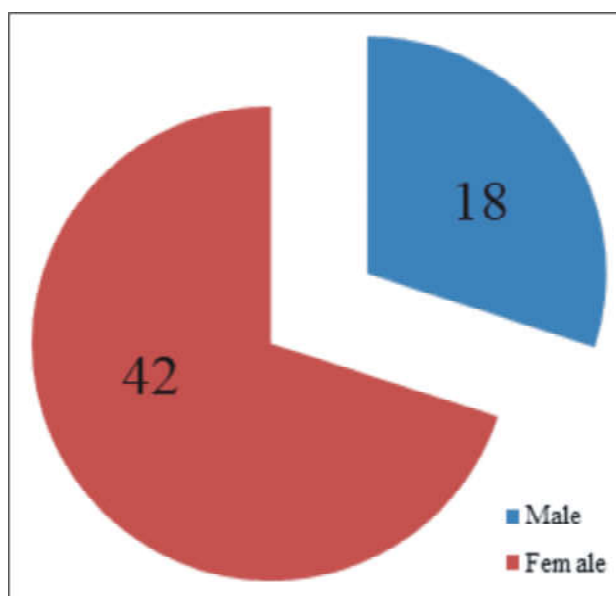

Fig. 1: Gender distribution.

The age and sex distribution of patients with RA is shown in Fig. 2.
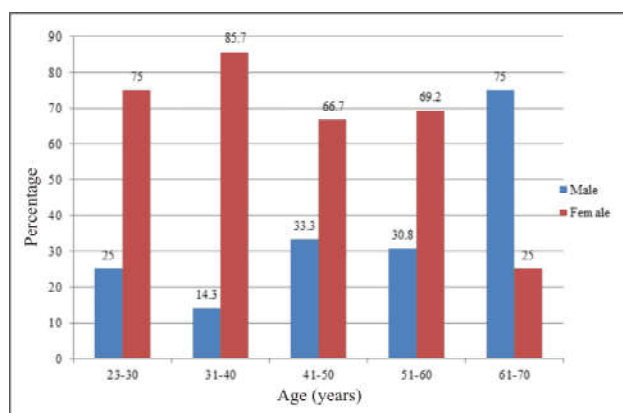
Fig. 2: Age distribution with sex.

In the study group, total echocardiographic abnormalities were found in 21 patients out of 60 cases and the maximum incidence was between 24 - 60 years (90.47%) age group. The oldest patient was 62 years and the youngest was 24 years. The mean age group was 43.83 with standard deviation of 11.01 years. The disease was found to be more common in females with the female: male ratio being 2.3: 1 (7 : 3). Cardiac abnormalities were seen maximum in 41 - 50 years age group (Table I).

Table I: Age and sex distribution in Rheumatoid arthritis patients with echocardiography abnormality.

Age groups	No. of cases (%)	Male (%)	Female (%)	Echocardiography abnormality (%)	ECG
20 - 30	8 (13.3)	2 (25)	6 (75)	1	LAHB, Non sp ST-T
31 - 40	14 (23.3)	2 (14.3)	12 (85.7)	3	–
41 - 50	21 (35)	7 (33.3)	14 (66.7)	10	Incomp RBBB, Non sp ST-T
51 - 60	13 (21.7)	4 (30.8)	9 (69.2)	5	LVH, Non sp ST-T, RBBB
61 - 70	4 (6.7)	3 (75)	1 (25)	2	LVH
Total	60 (100)	18 (30)	42 (70)	21	–

The duration of the disease ranged from 1 year to 20 years and the mean duration being 4.55 years with a standard deviation of 3.52. In general, cardiac abnormalities were more common in patients with longer duration of illness (Table II).

Discussion

Cardiac involvement is a well-documented extra-articular manifestation in autopsy studies. These abnormalities have also been documented in several studies using echocardiography and Doppler analysis. In this study cardiac structural and functional abnormalities were seen in 21 patients. Left ventricular filling abnormalities were the most common and were seen in 13 (21.6%) patients. Pericardial involvement was seen in 6 (10%) patients of whom 4 (6.66%) patients had pericardial effusion and 2 (3.33%) patients had pericardial thickening. One patient with pericardial thickening was also found to have aortic root dilatation. Pulmonary hypertension was seen in 4 patients (6.66%), and tricuspid regurgitation were present in one patient. Mitral regurgitation was seen in 3 patients (5%) of total RA cases.

Table II: Duration of disease among RA patients with echocardiographic abnormalities.

Duration of disease (years)	No. of RA patients	No. with Echocardiographic abnormalities	Specifics
1 - 2	11	4	LV dysfunction, PAH, pericardial effusion
>2 - 3	14	2	LV dysfunction, pericardial thickening + aortic root dilatation
> 3 - 4	16	7	LV dysfunction, MR, PAH, pericardial effusion, pulmonary hypertension (PAH), TR
> 4 - 5	7	2	LV dysfunction
> 5 - 10	8	5	LV dysfunction, pericardial effusion
> 10	4	2	LV dysfunction, MR, PAH

Maione *et al* found the incidence of cardiac involvement to be 43% in their study and the most common abnormality was LV diastolic dysfunction (26%)[5].

Corrao *et al* in 1996 evaluated 40 patients for ventricular function in patients of RA and found that ventricular filling abnormalities were significantly higher in RA patients[6].

Levendoglu *et al* in 2002 evaluated forty patients with rheumatoid arthritis for ventricular function using Doppler echocardiography. They found a significantly increased incidence of left ventricular diastolic dysfunction[7].

The mean age of patients with RA in this study was 43.83 ± 11.011 years with a range of 24 to 62 years. Maximum patients were found with cardiac abnormalities[10] in 41 - 50 years age group. The maximum incidence of cardiac abnormalities (50%) was found in the 61 - 70 years age group. The female to male ratio in this study was 2.3: 1.0.

In the study by Kaushal *et al*, the median age was 29 years with a range of 14 - 56 years, and the male to female ratio was 1:5. The age group in this study was similar to that of the present study[8].

In this study there was significantly higher incidence of cardiac involvement in patients with increased disease duration. The mean duration of disease in patients was 4.55 ± 3.52 years and the mean duration of disease in patients presenting with cardiac abnormalities was 5.07 ± 3.23 years. The mean age of patients with LV diastolic dysfunction was found to be 4.61 ± 2.85 years.

Franco et al evaluated 32 patients of RA to assess LV diastolic abnormalities by Doppler echocardiography. They found a direct relationship with a linear increase in incidence of left ventricular diastolic dysfunction with increasing duration of disease[9]. The present study also had similar findings with an increasing duration of disease in patients with left ventricular filling abnormalities.

The extra-articular manifestations noted apart from cardiac abnormalities were rheumatoid nodules in 5 patients, pleural effusion in 1 patient. In the present study there was significantly increased incidence of cardiac manifestations in patients with rheumatoid nodules (Odds ratio - 4.08, p < 0.001 S).

Wislowska et al evaluated echocardiographic findings in RA patients with subcutaneous nodules and compared them with RA patients without subcutaneous nodules. Their study revealed a significantly increased incidence of cardiac abnormalities in RA patients with subcutaneous nodules (odds ratio - 15, p < 0.0002)[10].

None of our 6 patients with LV filling defects had symptoms or signs of cardiovascular disease. One female patient presented with chest pain on the right side which was dull aching, diffuse and a constant type of pain. On further evaluation this patient was found to have right-sided moderate pleural effusion which on investigation was found to be of rheumatoid aetiology. Pulmonary component of second heart sound was loud in one patient. This patient was found to have pulmonary hypertension with tricuspid regurgitation on echocardiography. Raised jugular venous pressure was found in one patient. This patient was found to have biventricular diastolic dysfunction.

The mean tender joint count in the present study in RA patients was 17.53 ± 5.39 in the entire group, in those without cardiac abnormalities 16.25 ± 5.52, and in those with cardiac abnormalities 19.9 ± 4.31. The mean swollen joint count was 7.96 ± 5.54 in the entire group of RA patients and 8.205 ± 6.03 in RA patients without cardiac abnormalities. The mean swollen joint count in RA patients with cardiac abnormalities was 7.52 ± 4.6.

There was significant correlation (p = 0.01 S) between the tender joints count and occurrence of cardiac abnormalities; however, there was no correlation between swollen joints count and incidence of cardiac lesions (p = 0.65 NS).

The mean ESR in the study group was 52.25 ± 24.17 mm/hr and ranged from 12 mm to 100 mm/hour. The mean ESR in RA patients without cardiac abnormalities was 50.71 ± 25.32 mm/hr, and in those with cardiac abnormalities 55.09 ± 22.205 mm/hr. Patients with cardiac abnormalities were found to have higher ESR values; however, the association was not found to be statistically significant (p = 0.5 NS).

The rheumatoid factor was positive in 85.7% of patients with cardiac abnormalities as compared to 69.23% in RA patients without cardiac abnormalities. This study shows mean RA factor 81.57 ± 31.02 mm/hr in patients with cardiac abnormalities and mean RA factor 75.15 ± 24.48 mm/hr in patients without cardiac abnormalities (p = 0.38). Hence, patients with cardiac abnormalities were found to be more likely to be seropositive; however, this correlation was not found to statistically significant (p = 0.38).

The CRP was positive in 51.66% in RA patients. This study shows mean CRP 3.95 ± 2.63 mg/dl in patients with cardiac abnormalities and mean CRP factor 2.407 ± 1.11 mg/dl in patients without cardiac abnormalities (p = 0.002 S).

The ACPA was positive in 70% in RA patients. This study shows mean ACPA 237.39 ± 76.04 unit/ml in patients with cardiac abnormalities and mean ACPA 247.85 ± 39.33 unit/ml in patients without cardiac abnormalities (p = 0.89 NS)[11].

X-ray of the hands were taken for all RA patients and were staged under Steinbrocker's radiological classification. There was significant association of cardiac abnormalities with higher stages of Steinbrocker's radiological classification.

Dawson et al found that cardiac abnormalities were found to occur more commonly in patients with erosive arthritis[12].

Two patients were found to have left ventricular hypertrophy as per the Romhilt Estes criteria. This patient was found to be having hypertension and was on regular treatment since last 1 year. Echocardiography revealed left ventricular diastolic dysfunction with increase in the LV muscle mass. Other ECG changes were nonspecific ST-T changes and minor conduction abnormalities which were comparable to the ECG findings in the control group[13].

Echocardiography was done in all the 60 RA patients. Structural and functional abnormalities were found in 21 RA patients. Left ventricular diastolic dysfunction was found to be the most common abnormality (21.6%). One patient was found to have biventricular diastolic filling abnormality. Decreased E/A ratio and increased S/D ratio were suggestive of decreased ventricular filling which was evident in Doppler echocardiography especially in the early part of diastole. However, there was no change in the left atrial indices or gross reversals in pulmonary flow suggesting that the LV dysfunctions were of mild-to-moderate grade.

Among all the ventricular dysfunction patients, only one had increase in LV mass and comparable to that of the controls. This patient was found to be hypertensive and had been on regular anti-hypertensive treatment since 1 year. In the remaining patients with LV dysfunction in the absence of change in the LV mass, the diastolic filling abnormalities were suggestive of a reduction in ventricular elasticity and an intrinsic myocardial abnormality. Autopsy studies have shown non-specific myocarditis, granulomatous lesions, coronary in 1% of patients only. This is comparable with the low incidence vasculitis, secondary amyloidosis and diffuse fibrosis are histopathological features in patients with RA[14].

None of our patients were found to have any evidence of ischemic heart disease as a possible cause for the diastolic dysfunctions. Pericardial involvement was seen in 6 patients. Four patients were found to have pericardial effusion. The effusion in both cases was small (< 300 ml) and hence not associated with any ventricular dysfunctions, or significant clinical manifestations. Two patients were found to have pericardial thickening, and aortic root dilation was seen in 1 patient.

Four patients were found to have pulmonary hypertension, and three patients had mitral regurgitation out of 60 RA patients. Pulmonary hypertension with tricuspid regurgitation was seen in 1 female patient.

Conclusion

Cardiac manifestations were seen in 21 patients (35%) out of 60 cases studied the maximum incidence of the disease was seen in the 41 - 50 years age group and the female to male ratio was 2.3: 1.0. The mean duration of the disease was found to be 4.55 ± 3.52 years in the entire study group and 5.07 ± 3.23 years in patients presenting with cardiac abnormalities. There was positive correlation of patients with cardiac abnormalities to tender joints count, subcutaneous nodules, erosive arthritis and mean duration of disease, C-reactive protein. However, there was no significant correlation between cardiac abnormalities and swollen joints count, ESR, seropositivity, functional class and anti-CCP antibody. Most patients with cardiac abnormalities had normal chest X-ray. The electrocardiogram revealed minor conduction abnormalities in patients.

The most common cardiac abnormality seen in this study was left ventricular diastolic dysfunction in 21.6%. Other abnormalities included pericardial disease in 10%, pulmonary hypertension in 6.66%, mitral regurgitation in 5%, and tricuspid regurgitation in 1.66%.

None of the patients had clinical manifestations of cardiac involvement, suggesting that cardiac involvement in rheumatoid arthritis is a sub-clinical disease.

Summary

Cardiac abnormalities are an important extra-articular manifestation of rheumatoid arthritis. These abnormalities are largely sub-clinical. The early detection of cardiac abnormalities can be very important in the correct assessment and management of the RA patients, especially in light of the fact that the most common cause of mortality in RA patients is cardiovascular disease. Therefore, every patient should be submitted to a cardiological assessment (in particular echocardiography) in order that cardiac involvement can be detected early and treated, and the incidence of morbidity and mortality reduced.

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ORIGINAL ARTICLE

Behavioural Etiquettes of Visitors attending the Respiratory Diseases and Swine Flu OPD in Relation to Droplet Borne Infection and its Correlation with Age, Sex and Education Status: a Prospective Observational Study

Manoj Saluja*, Vishwas Galav**, Saurabh Chittora***, Drishya Pillai**, Sidharth Sharma**

Abstract

Introduction: Air-borne droplet infection is a major source of transmission of several respiratory infections such as common influenza, swine flu, pneumonia, tuberculosis, etc., and demands strict practices, i.e., face-mask, etc., on the part of patient and community at large to avoid spread of disease. However behaviour of members of the community is generally not in accordance with norms.

Objective: To determine the behavioural etiquettes of visitors attending swine flu OPD and the level of hygiene maintained by them while coughing, we conducted a prospective observational study at GMC, Kota, involving 696 participants including both sexes and age more than 10 years attending the "Seasonal respiratory diseases and swine flu OPD".

Material and Methods: Patients attending the "Seasonal respiratory diseases and swine flu OPD" were observed for behavioural pattern and the level of hygiene maintained by them while coughing.

Primary predefined observational outcomes include: 1) Coughing with face covered by a cloth or handkerchief, 2) Coughing with face covered by hand, 3) Coughing without any protection in open air.

Observation: A large section of study population (n = 591, 84.91%) were not using a cloth or handkerchief to cover their mouth while coughing. Some participants covered their mouth with bare hands while coughing (n = 300, 43.1%) while maximum participants did not use any sort of protection while coughing (n = 291, 41.8%). Further, behaviour aletiquettes have a significant relationship with sex and education status of the participants but not their age.

Conclusions: The number of visitors not covering their face with a cloth/handkerchief while coughing is 84.91% which is a huge contributor to the spread of fatal infections such as swine flu via the air-borne route. Health and hygiene education is a simple and cost-effective method to prevent such infections.

Introduction

Air-borne droplet infection is a major source of transmission of several respiratory infections such as common influenza, swine flu, pneumonia, tuberculosis, etc., and demands strict practices on the part of patients and the community at large to avoid spread of disease, i.e., face-mask, etc. However, behaviour of members of community is generally not in accordance with norms. We did a observational study of 696 unselected individuals to analyze the behavioural pattern while coughing among the patients and general population attending the "seasonal respiratory diseases OPD" to assess the behavioural etiquettes followed by them which will further enhance our efforts in prevention of respiratory diseases. We further tried to assess whether certain factors such as age, sex, and education status of the patients had any significant effect on the level of hygiene maintained by them while coughing.

Objective

To determine the behavioural etiquettes of visitors attending the "seasonal respiratory diseases and swine flu OPD" and the level of hygiene maintained by them while coughing, and its relation to their age, sex, and education status, we conducted a prospective observational study at Govt. Medical College, Kota, involving 696 participants including both sexes and age more than 10 years.

Material and methods

Patients attending the "Seasonal respiratory diseases and swine flu OPD" were observed for behavioural pattern and the level of hygiene maintained by them while coughing.

Adequate warning signs were placed outside and inside the OPD and at various places in the hospital premises to raise awareness of the patients regarding the signs and

*Senior Professor, **Resident, ***Assistant Professor, Department of Medicine, Government of Medical College, Kota - 324 010, Rajasthan.
Corresponding Author: Dr Vishwas Galav, Resident, Department of Medicine, Government Medical College, Kota - 324 010, Rajasthan. Tel: 8619057146, E-mail: vishu28galav@gmail.com.

symptoms, prevention and precautions for swine flu as shown below.

Image 1: Precautions for acute influenza and swine flu.

Image 2: Signs and symptoms of swine flu.

Primary predefined observational outcomes include:- 1.) Coughing with face covered by a cloth or handkerchief; 2.) Coughing with face covered by hand; 3.) Coughing without any protection in open air.

A questionnaire consisting of the age, sex and educational status of the patient, and one of the three predefined

Image 3: Preventive measures for caregivers of swine flu patients.

observation outcomes was prepared. Any patient or visitor attending the OPD who was found to be couging was observed for the pattern of coughing and segregated on the basis of above three variable outcomes. The rest of the details were noted down as per the questionnaire.

Questionnaire

- Patient name: Age:
- Sex: Place:
- Educational status (encircle)
  Illiterate   Primary   Secondary   College/Degree
- Cover face while coughing (encircle) (as observed by attending physician)
  Yes (with handkerchief)   Yes (with hand)   No

- Aware of preventive health benefits of covering face while coughing

  Yes   No

Study subjects

696 unselected individuals (age > 10 years; 315 females and 381 males) with acute seasonal respiratory illness, attending the respiratory diseases and swine flu OPD at Govt. Medical College, Kota were included in the study.

Inclusion criteria

Included all patients and visitors coming to OPD with cough and willingness to participate in a research study. Without exception, all participants completed the questionnaire.

Exclusion criteria

Patients aged less than 10 years.

No physical or biochemical measurements were required. Post-observation, information was given to the patient about the objective of study.

Statistical analysis

Qualitative variables were expressed as percentages. The comparisons of qualitative variables were performed using Pearson's chi-squared test. A value of $p > 0.05$ is considered as not significant, $p < 0.05$ as significant thus giving a 95% confidence interval (CI).

For comparison of coughing pattern in relation to age, patients were divided into three age groups, i.e. Young (11 - 30 years), middle-aged (31 - 60 years) and elderly (> 60 years).

For comparison of coughing pattern in relation to educational status, patients were divided into four categories, i.e., Illiterate, elementary education (1st - 8th standard), secondary education (9th - 12th standard), college educated (attended college: diploma/degree).

For comparison of coughing pattern in relation to sex, patients were divided into two categories, i.e., male and female.

Results

A large section of the study population (n = 591, 84.91%) were not using a cloth or handkerchief to cover their mouth while coughing. Some participants covered their mouth with bare hands while coughing (n = 300, 43.1%) while maximum participants did not use any sort of protection while coughing (n = 291, 41.8%).

Relation with age: The following data were obtained in
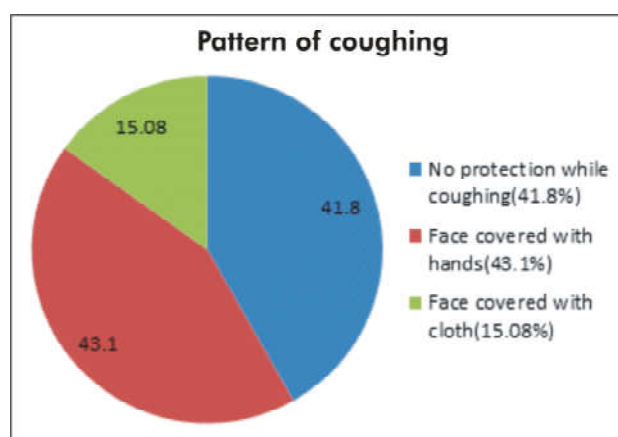
Fig. 1: *A pie chart showing the percentage distribution of people with different behavioural etiquettes while coughing.*

various age groups.

Table I: A data table showing percentage distribution of people in different age groups in relation to their behavioural etiquettes while coughing.

Age group	Face covered with cloth while coughing	Face covered with hand while coughing	Face not covered while coughing	Total
Young (11 - 30)	54 (14.75%)	174 (47.54%)	138 (37.7%)	366 (100%)
Middle-age (31 - 60)	45 (17.04%)	105 (39.77%)	114 (43.18%)	264 (100%)
Elderly (> 60)	12 (18.18%)	21 (31.81%)	33 (50%)	66 (100%)

Only 37.7% participants of younger age did not cover their face at all while coughing as compared to 43.1% participants of middle-age group and 50% in older age group. The above data suggests that people of younger age group were more likely to cover their face while coughing as compared to elderly counterpart (62.89% versus 49.99%). The middle-age group people are also more likely to cover their face while coughing as compared to the elderly (56.81% versus 49.99%). However, this correlation is not statistically significant ($p > 0.05$).
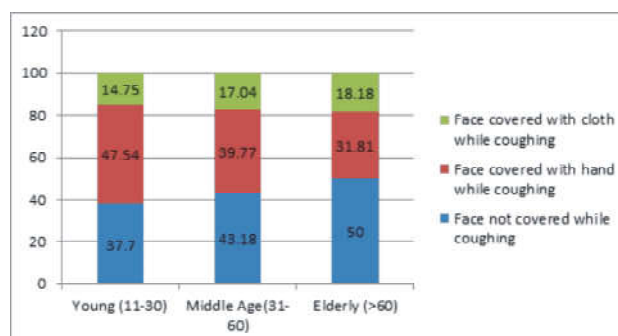
Fig. 2: *A bar graph showing the relationship of age of people with behavioural etiquettes while coughing.*

Relation with sex: The following data were obtained.

Table II: A data table showing percentage distribution of people with both sexes in relation to their behavioural etiquettes while coughing.

Sex	Face covered with cloth while coughing	Face covered with hand while coughing	Face not covered while coughing	Total
Male	36 (9.44%)	168 (44.09%)	177 (46.45%)	381 (100%)
Female	66 (20.95%)	132 (41.90%)	117 (37.14%)	315 (100%)

- 20.95% female covered their face with a cloth as compared to only 9.44% male.

- 37.14% female did not cover their face while as compared to 46.45% males suggesting that females are significantly more likely to cover their face while coughing than males (p < 0.05).
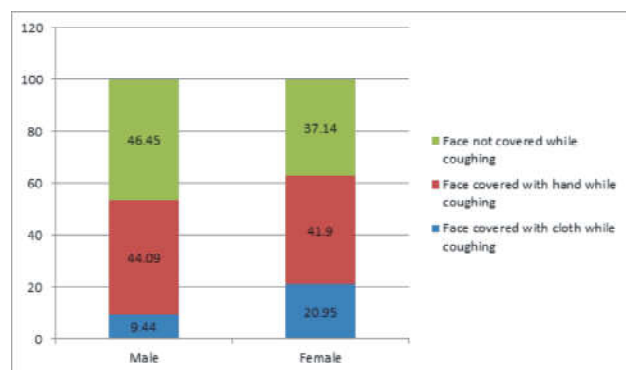
Fig. 3: A bar graph showing the relationship of sex of people with behavioural etiquettes while coughing.

Relation with education status

The following data were obtained in various age groups.

Table III: A data table showing percentage distribution of people with different educational status in relation to behavioural etiquettes while coughing.

Education status	Face covered while coughing	Face not covered while coughing	Total
Illiterate	81 (49.09%)	84 (50.90%)	165 (100%)
Elementary education	129 (60.56%)	84 (39.43%)	213 (100%)
Secondary education	93 (59.61%)	63 (40.38%)	156 (100%)
College educated	114 (70.37%)	48 (29.62%)	162 (100%)

- College educated participants are the most likely to cover their face (70.37%) while coughing, and illiterate participants are the least likely (49.09%). Primary and secondary educated participants are intermediate between the two groups (60.5 and 59.6% respectively).

- This shows that there is strong statistical association of

educational status of an individual and his behavioural hygiene pattern in relation to coughing in public (p < 0.05).
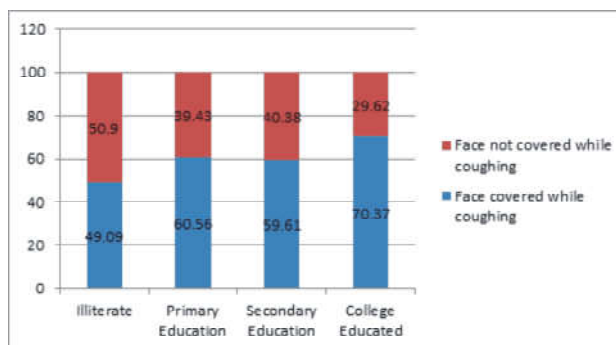
Fig. 4: A bar graph showing the relationship of educational status of people with behavioural etiquettes while coughing.

Awareness of population regarding role of behavioural hygiene (covering face while coughing) in preventing transmission of air-borne/droplet respiratory infections.

Table IV: A data table showing percentage distribution of people regarding their awareness of prevention methods regarding droplet infection while coughing.

Awareness	Male	Female	Total
Present	317 (83.20%)	280 (88.88%)	597 (85.77%)
Absent	64 (16.80%)	35 (11.11%)	99 (14.79%)
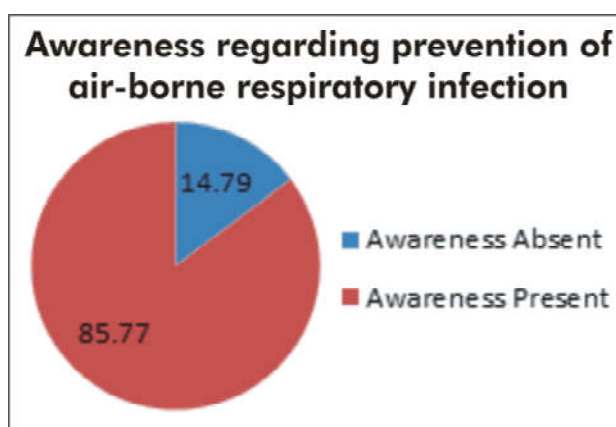
Fig. 5: A Pie chart showing the percentage distribution of people regarding their awareness of prevention methods regarding droplet infection while coughing.

Discussion

Communicable viral respiratory diseases may be transmitted through various routes, including airborne, direct and indirect contact, and droplet transmission. Droplet and contact transmission are traditionally defined as requiring

close contact to occur, whereas airborne transmission may occur over much larger distances. As such, transmission of natural infection is seen over long (greater than 1 m between source and susceptible individual) and shorter (less than 1 m between source and susceptible individual, such as during a casual conversation) distances, for those agents spread via the airborne route. In other words, a hallmark of airborne agents is that they should result in infections at long distance from the source. Few, if any, respiratory viruses are thought to be exclusively transmitted via one route. To show the significance of a particular route, other potential confounding routes must be considered when designing experiments and drawing conclusions from epidemiological data. There is no exact particle size cut-off at which pathogen transmission changes from exclusively droplet to airborne, or vice versa. Rather, as particle sizes decrease below 5 µm, droplet transmission presumably blends into airborne transmission[1].

The precise mechanism of transmission of respiratory tract infections in man is of great practical importance since control measures may be specific for a single mechanism. For example, droplet nuclei can be controlled by technique of air disinfection which does not affect larger respiratory droplets. Control of spread by air disinfection thus identifies the infection as droplet nucleus-borne. Without denying the possibility of transmission of some respiratory tract infections by larger droplets[2,3], we subscribe to the hypothesis that droplet nuclei are primarily responsible for epidemic spread of respiratory tract infections in the community[4].

Common droplet borne infections include:-

1. Meningococcal meningitis
2. Pertussis
3. Mumps
4. Rubella
5. Streptococcal pharyngitis
6. Influenza
7. Coronaviruses (SARS, MERS, n-COV 2019)

In humans, seasonal influenza viruses are a significant cause of morbidity and mortality and constitute an economic burden of $10.4 billion dollars per year in the USA[5]. Several studies have demonstrated the utility of hygiene in controlling respiratory infections.

Table V:

Author/country/ year of exposure/ (reference)	Study design and participants	Reported results
Aiello/USA, 2006/07[7]	Cluster parallel randomisation of 1,437 students living in university residence halls to 3 arms and analysed as control group (552 students); mask plus hand sanitiser group (367 students); and mask only group (378 students); instructed to wear mask as much as possible in residence hall during 6 week intervention period; encouraged to wear outside residence hall also. Outcome measure: self reported ILI.	Adjusted analyses found ILI significantly reduced in mask plus hand sanitiser hygiene group compared with controls (during weeks 4 - 6), ranging from 35% (95% CI 9 - 53%) to 51% (95% CI 13 - 73%); reductions in the mask group not significant at $P < 0.025$.
Shuangsheng Wu et al/China, 2015/16[6]	A multi-stage sampling, cross-sectional survey of adults living in Beijing using self-administered anonymous questionnaires	After adjusting for demographic characteristics, the variables significantly associated with a lower likelihood of reporting ILI were regular physical exercise (OR 0.80; 95% CI 0.74 - 0.87), optimal hand hygiene (OR 0.87; 95% CI 0.80 - 0.94), face mask use when going to hospitals (OR 0.87; 95% CI 0.80 - 0.95), and not sharing of towels and handkerchiefs (OR 0.68; 95% CI 0.63 - 0.73).
Cowling BJ et al/ Hong Kong, 2008/09[8]	To investigate whether hand hygiene and use of facemasks prevents household transmission of influenza. Lifestyle education (control) (134 households), hand hygiene (136 households), or surgical facemasks plus hand hygiene (137 households) for all household members of rapid test positive influenza patients.	In 154 households in which interventions were implemented within 36 hours of symptom onset in the index patient, transmission of RT-PCR confirmed infection seemed reduced, an effect attributable to fewer infections among participants using facemasks plus hand hygiene (adjusted odds ratio, 0.33 [95% CI, 0.13 to 0.87]).

In this observation-based study we aimed to study the behaviour of people in relation to the level of maintenance of hygiene while attending OPD. We found that very few people (14%) follow the required level of hygiene while coughing in public places. We further tried to correlate various factors such as age, sex, and educational status of study participants in relation to their level of hygiene while coughing in public.

We found that age has no statistically significant correlation with level of hygiene maintained while coughing in public.

Sex and educational status of participants are significantly correlated with the level of hygiene. Females are more likely to cough with mouth covered as compared to males. People with higher educational status are more likely to cover their face while coughing – with a highly significant difference between illiterate and highly educated people. While 85.7% of the population was aware of the utility of covering the face while coughing in preventing respiratory tract infections, only 59.9% population was actually covering the face with either a cloth or a handkerchief, and only 14.8% population was using a cloth.

The high level of hygiene observed by the more educated people could in turn lead to significant decline in transmission of airborne respiratory diseases like swine flu and newly spreading novel coronavirus 2019. While further studies on a larger scale are required to determine the exact number of cases that can be prevented by educating the people on public behavioural hygiene, we can conclude form this study that more educated people are less likely to transmit airborne diseases in public spaces as compared to less educated ones and educating people on public hygiene can drastically reduce the burden of communicable respiratory diseases in the community.

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ORIGINAL ARTICLE

Occurrence and Natural Course of Ceftriaxone-associated Nephrolithiasis in Indian Children – A Prospective Observational Study

Dheeraj Bahl*, Varsha Jayan**

Abstract

Objective: To study the occurrence and natural course of ceftriaxone-associated nephrolithiasis in Indian children.

Study design: Prospective observational study.

Participants: 153 children who received intravenous ceftriaxone during hospitalisation.

Intervention: A total of 153 children who received intravenous ceftriaxone during hospitalisation were enrolled in our study. Serial ultrasonography was performed to look for appearance and disappearance of renal calculi. Children who developed renal calculi were followed-up for symptoms of renal calculi.

Results: The incidence of nephrolithiasis after ceftriaxone therapy was found to be 1.3% (2 out of 153). The incidence of renal calculi was equal in both genders. In our study we did not find any significant relationship between occurrence of nephrolithiasis with age and dose of ceftriaxone. Size of renal calculi in both cases was almost similar, i.e., 2 mm and 3 mm. We observed that, in both cases renal calculi occurred after 7 days of ceftriaxone. Disappearance of renal calculi was seen after 8 weeks and 6 months of stopping ceftriaxone. No symptoms of renal colic were observed during follow-up in both cases.

Conclusion: Ceftriaxone associated nephrolithiasis is seen to be reversible. Creating awareness among surgeons can avoid unwanted surgery in such cases as these patients can be managed conservatively.

Introduction

Nephrolithiasis is one of the most commonly known ancient (since 4000 BC) ailment affecting the urinary tract of human beings. Approximately 12% of the global population is afflicted by nephrolithiasis at any time during their life span. The risk of developing urolithiasis in adults appears to be higher in the western world (5 - 9% in Europe, 12% in Canada, 13 - 15% in the USA) than in the eastern hemisphere (1 - 5%), although the highest risks have been reported in some Asian countries such as Saudi Arabia (20.1%). The incidences of urinary bladder stones is decreasing in developed countries but are frequent during the first years of life in various areas of Turkey, Iran, India, China, Indochina and Indonesia[1].

The various factors influencing the formation of nephrolithiasis include gender, dietary habits nutritional status, tropical regions – probably due to scant fluid intake and low urinary output[2]. Probably better modalities of diagnosis and treatment are also a contributing factor. In Indian population, approximately 12% are expected to have urinary stones, and out of these 50% may end up in renal failure[3].

Overview of risk factors

In children with nephrolithiasis, an underlying risk factor is identified in 75 to 85 per cent of affected children[8-12], e.g., urinary tract infection, and/or a structural renal or urinary tract abnormality[4-9].

Drug-induced nephrolithiasis is rarely described in children. The incriminated drugs are ceftriaxone, trimethoprim-sulfamethoxazole, topiramate, antiretroviral drugs (atazanavir, indinavir, sulfadiazine)[10].

Ceftriaxone is one of the third-generation parenteral bactericidal cephalosporin, is widely used across the world in the treatment of various infections in patients including children. On account of its long plasma half-life, it can be used as once-a-day dosage.

Schaad *et al* in 1988 for the first-time reported association of ceftriaxone and biliary pseudolithiasis[11]. Cochat *et al* in the year 1990 reported ceftriaxone-induced nephrolithiasis for the first-time and it was predominantly seen in cases of treatment of meningitis and diarrhoea who were put on ceftriaxone[12]. In both the conditions there was probably compromise of intravascular fluid.

*Professor,**Post-Graduate Student, Department of Paediatrics, Atal Bihari Vajpayee Institute of Medical Sciences (ABVIMS) and Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 110 001.
Corresponding Author: Dr Varsha Jayan, Professor, Department of Paediatrics, Atal Bihari Vajpayee Institute of Medical Sciences (ABVIMS) and Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 110 001. Tel: 9497273697, E-mail: varshajayan@yahoo.co.in.

Chutipongtanate *et al* showed that ceftriaxone at therapeutic levels could be crystallised with free calcium in the urine under physiologic conditions. He hypothesizes that tubular occlusion and crystal-cell adhesion may play an important role in the pathogenic mechanisms of ceftriaxone-induced nephrolithiasis[13].

The preparation used for the intravenous route is ceftriaxone disodium salt, which is poorly water-soluble[14]. Avci *et al* in 2003 suggested that ceftriaxone can bind with calcium ions, producing a precipitate that forms biliary sludge, also known as biliary pseudolithiasis. The same has also been observed in many studies since 1988[15].

Wang *et al* in 2017 observed that complications associated with ceftriaxone treatment, such as gallstonea and nephrolithiasis do happen[16] while Arvidsson *et al* elaborated that approximately 33 - 67% of the administered dose is eliminated through renal excretion, while 40% is secreted in the bile[17].

There is no information on urinary tract calculi as one of the side-effects of ceftriaxone in Indian children. Also, no study has been carried-out in ceftriaxone induced nephrolithiasis in India. In this light, the present study was performed to assess the incidence of nephrolithiasis and gallstones associated with ceftriaxone administration, possible correlation between ceftriaxone dosages, duration of treatment, weight of patient, diagnosis, BMI and age of the patient with nephrolithiasis, as well as any predisposing factors in Indian children on ceftriaxone.

Material and methods

This was a prospective observational study conducted after ethical clearance, in the department of Paediatrics and Radiodiagnosis in a tertiary care teaching hospital in New Delhi, India, from November 2017 to March 2019. Any child admitted to this hospital with suspected or definite bacterial infection was eligible for the study if the physician in charge of the case had decided to start ceftriaxone therapy. A total of 153 patients who received intravenous ceftriaxone (same dosage and brand) during hospitalisation were enrolled in our study. The clinical conditions for which ceftriaxone was given included – meningitis, infective diarrhoea, enteric fever, peritonitis, cellulitis, liver abscess, respiratory tract infections.

For each case, routine investigations including haemogram, LFT, KFT, urine analysis, urine culture before and after treatment, serum levels of calcium, urea, and creatinine, spot urine levels of calcium and creatinine were sent.

There was no fluid restriction and dehydration were monitored clinically and prevented.

Ultrasonography was done to assess renal, ureteric, and urinary bladder calculi, biliary sludge, cholelithiasis, and calculi size. USG was done on D0, D3, D7, D21 and further follow-up on D45, D60, till the findings disappeared. Ultrasonography on all the enrolled patients were done using the same equipment and the presence of nephrolithiasis was confirmed by two operators.

Criteria for positive renal calculi were bright echogenic foci with posterior acoustic shadowing while for gallstones presence of mobile, gravity dependent, echogenic material accompanied by clear acoustic shadowing and sludge was diagnosed when hyperechogenic bile showed no acoustic shadowing. Patients who developed renal calculi, biliary sludge/calculi were followed-up for symptoms of renal colic, acute cholecystitis like fever, vomiting, right hypochondrial pain. The respective days of appearance and disappearance of renal calculi, biliary sludge, cholelithiasis were noted. Persistence of either stone or sludge beyond 60 days was to be followed-up to 6 months or disappearance whichever is earlier. For each patient, we calculated the urine calcium: creatinine ratio before and after treatment. The means for the two-time points were compared to assess the effect of ceftriaxone on urinary calcium excretion.

Exclusion criteria were congenital renal malformations, deranged renal function, those who already had renal calculi, underlying metabolic disorder, deranged LFT, KFT, underlying haemolytic anaemias, malabsorption or any chronic disease patients on drugs, e.g., furosemide, octreotide. sulfonamides, topiramate, antiretroviral.

Statistical methods

Descriptive statistics was analysed with SPSS version 17.0 software. Continuous variables were presented as mean ± SD. Categorical variables was expressed as frequencies and percentages. The Pearson's chi-square test or the chi-square test of association was used to determine if there is a relationship between two categorical variables.

The P value less than 0.05 was considered to be statistically significant.

Results

Only 2 patients out of 153 (1.3 %) showed the occurrence of renal calculi. The sizes were 2 mm and 3 mm each. In both the cases, renal calculi appeared after D7 of starting the CTX respectively. There was no occurrence of ureteric and urinary bladder calculi. Both were located on upper pole of kidney and disappeared after 8 weeks and 6 months respectively.

Table I: Correlation between renal calculi and age.

Age (years)	Renal Calculi		Total	p - value
	No	Yes		
< 3	43 (97.7%)	1 (2.3%)	44	–
3 - 6	41 (100.0%)	0 (0.0%)	41	0.998
6 - 9	33 (100.0%)	0 (0.0%)	33	0.998
> 9	34 (97.1%)	1 (2.9%)	35	0.870
Total	151 (98.7%)	2 (1.3%)	153	–

That is, there is no relationship between renal calculi and age. The table reveals that the occurrence of renal calculi is almost the same in all age groups.

Table II: Correlation between renal calculi and weight.

Weight (kg)	Renal Calculi		Total	p - value
	No (%)	Yes (%)		
< 10	18 (100.0%)	0 (0.0%)	18	–
10 - 20	81 (98.8%)	1 (1.2%)	82	0.999
20 - 30	40 (97.6%)	1 (2.4%)	41	0.999
30 - 40	12 (100.0%)	0 (0.0%)	12	1.000
Total	151 (98.7%)	2 (1.3%)	153	–

There is no relationship between renal calculi and weight. The table reveals that the occurrence of renal calculi is almost the same in all weight groups.

Table III: Correlation between renal calculi and height.

Height (CM)	Renal Calculi		Total	p - value
	No	Yes		
< 75	14 (100.0%)	0 (0.0%)	14	—
75 - 100	39 (97.5%)	1 (2.5%)	40	0.999
100 - 125	60 (100.0%)	0 (0.0%)	60	1.000
> 125	38 (97.4%)	1 (2.6%)	39	0.999
Total	151 (98.7%)	2 (1.3%)	153	—

That is, there is no relationship between renal calculi and height. The table reveals that the renal calculi is almost same in all height groups. Also, no correlation between BMI and renal calculi was noted.

Table IV: Correlation between renal calculi and dose.

Renal calculi	Mean	SD	Median	Mean rank	p - value
No	1890	747.7	1700	76.96	0.930
Yes	2050	1344	2050	79.75	

The table reveals that the dose of ceftriaxone is higher but not significant in cases with renal calculi (2,050 mg) compared to the cases with no occurrence (1,700 mg). There is no relationship between renal calculi and dose.

Table V: Correlation between renal calculi and duration of ceftriaxone.

Duration of ceftriaxone (Days)	Renal Calculi		Total	p - value
	No	Yes		
< 7	73 (100.0%)	0 (0.0%)	73	–
7 - 14	64 (97.0%)	2 (3.0%)	66	0.997
> 14	14 (100.0%)	0 (0.0%)	14	1.000
Total	151 (98.7%)	2 (1.3%)	153	–

Here the p-values suggest that the relationship between renal calculi and duration of ceftriaxone is not significant.

Table VI: Use of ceftriaxone in various infections.

Diagnosis	Renal Calculi		Total	p - value
	No	Yes		
CNS disease	28 (100.0%)	0 (0.0%)	28	–
Cardiovascular disease	10 (100.0%)	0 (0.0%)	10	1.000
Pulmonary disease	37 (100.0%)	0 (0.0%)	37	1.000
Gastrointestinal disease	60 (98.4%)	1 (1.6%)	61	0.998
Rheumatological and haematological disorders	8 (100.0%)	0 (0.0%)	8	1.000
UTI	8 (88.9%)	1 (11.1%)	9	0.998
Total	151 (98.7%)	2 (1.3%)	153	–

Here the p-values suggest that the relationship between renal calculi and diagnosis is not significant. That is, there is no relationship between renal calculi and diagnosis. The table reveals that the renal calculi are almost same irrespective of diagnosis.

None of our patients showed a change in urinary excretion of calcium related to ceftriaxone therapy.

Discussion

The objective of our study was to study the occurrence of ceftriaxone-associated renal calculi and its correlation with dose, duration, weight, height, BMI, infection type, and gender. Only two out of 153 patients developed renal calculi (1.3%). The occurrence of renal calculi was equal in males and females. This was similar in the study by Avci et al[15] and Ghodsiyeh Azarkar et al (1.5%) in 2013[24]. But Fesharakinia et al reported a higher incidence of (6.3%)

and all were asymptomatic[23].

In contrast Acun *et al*, showed higher occurrence in females[25]. Mohkam *et al*, too observed the incidence at 1.4%[22].

Size of renal stone in our study was 2 - 3 mm, whereas Mohkam *et al* reported a calculi size of 15.5 mm, and interpolar sized 6.5 mm.

The urine of children has high concentration of citrate and magnesium which inhibit formation of crystal. This may be a reason of infrequent nephrolithiasis as compared with adults[18,19].

No age, gender, race is exempt as far as occurrence of nephrolithiasis is concerned, but the occurrence and recurrence are higher in males in the age of 20 - 49 years.

Gender preponderance of nephrolithiasis is not seen in paediatric age group. Several studies do show a slightly higher prevalence in boys[20]. But a study by Huang *et al* found in Taiwan, a slightly higher prevalence in female children (52% versus 48%)[21].

Our study is consistent with studies carried out by Mohkam *et al* and Avci *et al* as far as occurrence of nephrolithiasis is concerned[22,15]. However, Fesharakinia *et al* in 2013 observed that there was a significant correlation between male sex and ceftriaxone related asymptomatic nephrolithiasis[23]. It was also observed that occurrence of ceftriaxone-related small sized nephrolithiasis at 6.3% was higher and one (1%) patient had gallbladder stone was on lower as compared to other studies[23].

In our study the occurrence of renal calculi is almost same in all age groups. It is in accordance with a study by Avci *et al*, in which the comparison of the groups with and without nephrolithiasis revealed no significant differences with respect to age of the patient[15].

The size of renal calculi in both cases (both located on upper pole of kidney) in our study was also same ranging from 2 - 3 mm. In both cases, renal calculi appeared on D7 after starting ceftriaxone, and disappeared after 8 weeks and 6 months respectively.

According to our study, there is no relationship between renal calculi and dose of ceftriaxone, this was in contrast to the study by Avci *et al* in 2004, who found that children receiving a high-dose ceftriaxone (100 mg/kg/day) for the treatment of severe infections developed small renal stones in 7.8% of cases[15].

In our study, there is no relationship between renal calculi and duration of ceftriaxone. The occurrence of renal calculi is almost same in all duration groups. Our study is in agreement with the study done by Avci *et al* which elaborated that paediatric patients may develop small sized,

asymptomatic renal stones during a 7-day course of ceftriaxone therapy[15]. Similar findings were noticed by Fesharakinia *et al* during a 2 - 6 day course of ceftriaxone therapy[23].

All the patients in our study remained asymptomatic and it is in accordance with a study conducted by Avci *et al* who also observed that the renal stones developed during a 7-day course of normal or high-doses of ceftriaxone therapy[15].

Prince JS, Senac *et al* and De Moor RA *et al* in their respective studies observed small size renal stones during a 2-6-day course of ceftriaxone and there was no significant difference in the two groups with and without nephrolithiasis as far as the dose and duration of therapy with ceftriaxone was concerned. Both the studies have reported high-doses of ceftriaxone and longer treatment time as risk factors for the development of nephrolithiasis[26,27].

It is important to understand occurrence of nephrolithiasis as complications of therapy with ceftriaxone as a self-limiting phenomenon. In contrast, confusion regarding these complications may lead to more invasive treatment such as surgery. There are many unreported incidences of surgical intervention in such ceftriaxone-induced pseudo-nephrolithiasis and pseudocholelithiasis. Awareness of ceftriaxone-induced pseudonephrolithiasis and cholelithiasis to the physician can certainly avert such eventualities and the patient can be managed conservatively.

Limitations

- In addition, the present study assessed the data from a single hospital; the findings of the research may not be more reliable than multicentre studies.

- Chemical composition of renal calculi could not be analysed due to non-availability of IR spectroscopy in our centre.

- It is difficult to make a proper conclusion in this study because of the small number of cases of nephrolithiasis arising out of ceftriaxone therapy.

Key messages

What is already known? Ceftriaxone is a broad-spectrum antibiotic that is widely used in the paediatric population. 60% of the drug is metabolised by the liver and the rest 40% by kidney. Cases of ceftriaxone-associated biliary cholelithiasis as well as nephrolithiasis have been reported. What does this study add? The occurrence of ceftriaxone-associated nephrolithiasis in our study has been found to be 1.3%. Our findings are similar to the previous studies

conducted on ceftriaxone-induced nephrolithiasis. But no study has been done in the Indian paediatric population. None of our patients developed symptoms of renal colic, and in both cases renal calculi resolved on follow-up. Hence, it is to be noted that ceftriaxone-associated nephrolithiasis can be managed conservatively without surgery.

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Rickets in Renal Tubular Acidosis: A Clinical Appraisal

Chhavi Agrawal*, Partha Pratim Chakraborty**

Abstract

Rickets, a metabolic disease restricted to an age group before epiphyseal growth plate fusion, is diagnosed by typical skeletal deformities and characteristic radiological features. The commonest aetiology of rickets worldwide is nutritional deficiency of vitamin D and/or calcium, followed by primary renal phosphate wasting disorders. Renal tubular acidosis is an important cause of rickets, particularly 'resistant rickets', as the diagnosis is often missed initially and the patients are being wrongly treated with other agents without any benefit. Renal tubular acidosis is characterised by normal anion gap metabolic acidosis and is classified into different subtypes. A systemic step-wise approach is needed in suspected patients to unveil the subtype of renal tubular acidosis and the underlying aetiology. Early diagnosis and proper management of renal tubular acidosis leads to complete clinical and radiological recovery in patients presenting with rickets secondary to renal tubular acidosis.

Key words: Rickets, renal tubular acidosis, urinary anion gap, tubular reabsorption of phosphate, tubular maximum for phosphate corrected for GFR.

Introduction

Rickets, a skeletal disorder limited to children and adolescents before epiphyseal fusion, is characterised by deficient mineralisation of the growth plate cartilages. The typical skeletal deformities and radiological abnormalities found in rickets are also associated with defective mineralisation of mature osseous matrix, a condition known as osteomalacia. Normal mineralisation of either the cartilages or the lamellar bone requires optimal calcium X phosphate product, which in turn depends on a homoeostatic system, finely regulated by vitamin D and parathyroid hormone (PTH). Three principal metabolic abnormalities found in overwhelming majority of children with rickets are defective vitamin D homoeostasis (deficiency, metabolism, and action), primary renal phosphate wasting, and calcium deficiency; hence rickets are often broadly classified as calciopenic rickets and phosphopenic rickets. While calciopenic rickets is secondary to calcium deficiency or altered vitamin D homoeostasis, phosphopenic rickets is the result of primary renal phosphate wasting, and is typically characterised by normal serum calcium and PTH[1,2]. However, it needs to be remembered that all forms of calciopenic rickets are associated with secondary hyperparathyroidism and resultant hypophosphataemia due to PTH-induced proximal renal tubular loss of phosphate. Hypophosphataemia, seen in both these forms of rickets, interferes with capase-9 mediated apoptosis of the hypertrophic chondrocytes, that ultimately gives rise to the typical clinical and radiological appearances.

Hypophosphatasia, a condition associated with deficient function of alkaline phosphatase (ALP) enzyme, chronic systemic acidosis due to any cause, and drugs like bisphosphonate, fluoride, aluminium, and parenteral iron are also associated with mineralisation defects of the cartilages and bones. Serum calcium and phosphate concentrations are usually normal in rickets secondary to these conditions. Two most common disorders associated with metabolic acidosis and rickets are chronic kidney disease and renal tubular acidosis (RTA) (Fig. 1).

Fig. 1: *A 4-year-old girl with rickets due to dRTA. Note the 'windswept' deformity (A) and wrist widening (D). Typical radiological features like cupping, splaying, fraying, and increased metaphyseal lucency are visible around the knee (B) and wrist joints (C).*

Renal tubular acidosis

RTA is a group of renal tubular disorders due to defects in proximal tubular reabsorption of bicarbonate ion (HCO_3^-), distal tubular excretion of hydrogen ion (H^+) or both, and is

*Resident, **Clinical Tutor, Department of Endocrinology and Metabolism, Medical College and Hospital, Kolkata, 88, College Street, Kolkata - 700 073, West Bengal.

Corresponding Author: Dr Partha Pratim Chakraborty, Department of Endocrinology and Metabolism, Medical College and Hospital, Kolkata, 88, College Street, Kolkata - 700 073, West Bengal. Phone: 9830092947, E-mail: docparthapc@yahoo.co.in.

characterised by hyperchloremic normal serum anion gap (AG) metabolic acidosis in patients with relatively normal glomerular filtration rate (GFR). Lost HCO_3^- in this condition is effectively replaced by chloride ion (Cl^-), resulting in hyperchloraemia and normal AG. Patients with estimated GFR (eGFR) between 20 - 50 ml/min/1.73M^2 usually have normal AG, while those with eGFR of < 20 ml/min/1.73M^2 have high AG. Acid-base disequilibrium in RTA occurs despite a normal or only mildly reduced glomerular GFR[3]. RTA is a poorly appreciated entity among many physicians, and understanding of the pathophysiology of the disease is important for subtyping and appropriate management. It can be classified into three major forms: type 1 or distal RTA (dRTA), type 2 or proximal RTA (pRTA), and type 4 or hyperkalemic RTA. dRTA is associated with reduced H$^+$ secretion, pRTA is characterised by impaired HCO_3^- reabsorption, and type 4 RTA is an acid-base disturbance generated by aldosterone deficiency or resistance. RTA can occur due to primary renal pathology or secondary to a variety of systemic diseases (Table I).

Table I: Types and aetiologies of RTA.

	Primary	Secondary
Distal RTA (type 1)	Sporadic or Hereditary (Mutation of H$^+$K$^+$ ATPase, H$^+$ATPase, AE1)	**Autoimmune:** Sjogren's, SLE, RA, PBC **Nephrotoxins:** Amphotercicn B, Trimethoprim, lithium **Miscellaneous:** Sarcoidosis, amyloidosis, obstructive uropathy
Proximal RTA (type 2)	Sporadic or Hereditary (Mutation of CA-IV, NHE-3, NBC-1)	**Autoimmune:** Sjogren's **Nephrotoxins:** tetracycline, topiramate, valproate, acetazolamide **Metabolic:** Wilson's disease, Cystinosis, Lowe's syndrome, Galactosaemia, chronic hypocalcaemia; Hereditary fructose intolerance, Tyrosinaemia **Miscellaneous:** Multiple myeloma, amyloidosis
Hyperkalemic RTA (type 4)	PHA-1, PHA-2 (Gordon's syndrome)	**Aldosterone deficiency or aldosterone resistance:** Hypoaldosteronism, ACEIs, ARBs **Hyporeninemic hypoaldosteronism:** Diabetes, Sickle cell disease Tubulointerstitial disease (eGFR: 20 - 50 ml/min) **Drugs:** Potassium sparing diuretics, NSAIDs, Trimethoprim, Pentamidine Cyclosporine, Tacrolimus
Mixed RTA (type 3)	Mutation in CA-II	Type 1 RTA with secondary proximal tubule dysfunction, Type 2 RTA with secondary distal tubule dysfunction

AE1: Anion exchanger 1; CA: Carbonic anhydrase; NHE-3: Sodium-hydrogen exchanger 3; NBC-1: Sodium-bicarbonate co-transporter 1; PHA: Pseudohypoaldosteronism; SLE: Systemic lupus erythematosus; RA: Rheumatoid arthritis; PBC: Primary billiary cirrhosis; ACE: Angiotensin converting enzyme; ARB: Angiotensin receptor blocker.

Free H$^+$, secreted from distal tubule constitute < 1% of total H$^+$ secreted; most protons are excreted as NH_4^+ (NH_3+H$^+$). dRTA is characterised by defective distal H$^+$ secretion, hence less urinary NH_4^+ excretion; as a result, urine pH is > 5.5, that is persistent and present during simultaneous systemic metabolic acidosis. Alkaline urine associated with hypercalciuria and hypocitraturia, often seen in dRTA, contribute to nephrocalcinosis and/or nephrolithiasis (Fig. 2)[4]. Serum potassium (K$^+$) is often low or normal, except when there is an underlying voltage-dependent defect, which is associated with impaired distal sodium (Na$^+$) transport and secondary impairment of distal K$^+$ secretion, leading to hyperkalaemia (hyperkalemic dRTA[5]. Hyperkalemic dRTA is different from type 4 RTA. In contrast to hyperkalemic dRTA, the ability to lower urine pH in response to systemic acidosis is maintained, and nephrocalcinosis is absent in type 4 RTA. Clinical manifestations in type 4 RTA are usually due to underlying disease, rather than RTA per se. An incomplete form of dRTA is often encountered, where patients demonstrate normal blood pH with low normal or mildly decreased serum HCO_3^- concentration, while lacking the ability to acidify urine when systemic acidosis is induced with an acidifying agent.
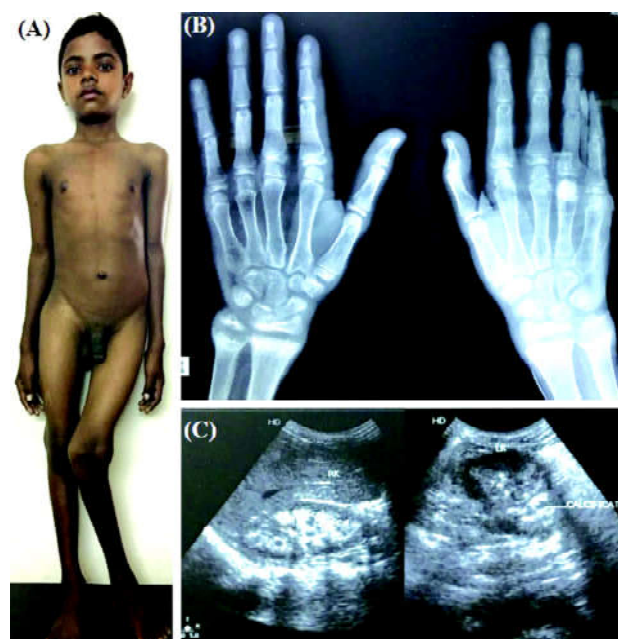
Fig. 2: dRTA in a 16-year-old boy with rickets and bilateral nephrocalcinosis (C). Metaphyseal changes are seen in B.

Proximal convoluted tubule (PCT) reabsorbs 80 - 85% of the filtered HCO_3^-, 10% is from the loop of Henle and remaining 5 - 10% is reabsorbed from collecting tubules. pRTA is characterised by impaired HCO_3^- reabsorption from PCT, i.e., a decrease in renal HCO_3^- threshold to 14 - 18 mmol/l, which is normally ~ 22 mmol/l in infants, and 25 -

26 mmol/l in children and adults[6]. Metabolic acidosis in pRTA tends to be milder because distal HCO_3^- reclamation remains intact and bicarbonaturia disappears when serum HCO_3^- concentration falls below the HCO_3^- tubular maximum (often at serum HCO_3^- level of 14 - 18 mmol/l). Urine pH in pRTA is variable; alkaline (> 5.5), if serum HCO_3^- concentration is above the threshold, and < 5.5 when serum HCO_3^- is below the threshold. pRTA may be isolated, or more commonly associated with Fanconi syndrome, a form of generalised proximal tubular dysfunction. Fanconi syndrome is a malabsorptive state of the PCT, wherein absorption of glucose, amino acids, low molecular weight proteins, phosphates, potassium, bicarbonate and uric acid are impaired; while pRTA refers to the deficiency in HCO_3^- retention only. Despite hypercalciuria, nephrocalcinosis/nephrolithiasis are infrequent, due to acidic urine and absence of hypocitraturia[7].

Type 3 RTA shares features of both type 1(dRTA) and 2 (pRTA). Carbonic anhydrase II (CA-II) deficiency, either inherited or acquired, presents with features of both pRTA and dRTA along with osteopetrosis, cerebral calcification and mental retardation due to deficiency of the enzymes in various organs[8]. Other conditions likely to be associated with type 3 RTA are acetazolamide use, Wilson disease, hereditary fructose intolerance and dysproteinemic syndromes. More commonly however, this pattern is observed as a transient phenomenon, when biochemical abnormalities arising out of dRTA (acidosis, hypokalaemia) induce proximal tubular dysfunction or metabolic alterations associated with pRTA (hypophosphataemia) impair distal tubular acidification mechanisms, thus contributing to a mixed phenotype of type 3 RTA[9,10].

In children and adolescents, RTA may present with failure to thrive, growth retardation, hypokalaemia, polyuria and polydipsia (due to defective urinary concentrating ability), nephrocalcinosis/nephrolithiasis (dRTA), and 'refractory rickets'. The definition of refractory rickets is not universally accepted; however, absence of radiological healing lines after 3 - 4 weeks of adequate calcium and vitamin D suggests non-nutritional rickets. An approach to such cases has been summarised in Fig. 3. Rickets and osteomalacia are common in dRTA and relatively uncommon in pRTA, unless associated significant acidosis and/or hypophosphataemia, as encountered in Fanconi syndrome. Features of rickets/osteomalacia are usually absent in incomplete dRTA and type 4 RTA unless the later is associated with uraemia.

Rickets in RTA

Rickets in RTA is multifactorial. Systemic acidosis is associated with defective mineralisation of the cartilages and bones due to increased solubility of the mineral phase. During acidosis, calcium and phosphate are mobilised from bones for the purpose of buffering by enhanced osteoclastic resorption. Enhanced osteoclastic activity results in influx of calcium and phosphate into the circulation. These molecules are subsequently lost through kidneys due to increased filtered load and reduced proximal tubular reabsorption secondary to systemic acidosis. Hypercalciuria results in secondary hyperparathyroidism that further aggravates hypophosphataemia due to renal phosphate loss. In addition, pRTA itself may be associated with phosphaturia and low renal 1α-hydroxylase activity, which leads to
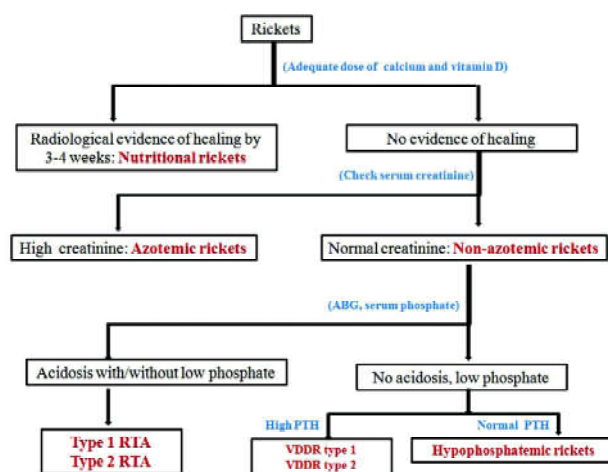
Fig. 3: Approach to refractory rickets.

impaired conversion of 25-hydroxy vitamin D to calcitriol (1, 25-dihydroxy vitamin D), the active form of vitamin D.

Approach

A thorough clinical survey of the child with rickets focussing the peripheral extremities, cranium, spine and eyes is of utmost importance. The authors recommend measurement of serum calcium, phosphate, albumin, ALP, PTH (by second generation assay), 25-hydroxy vitamin D, creatinine and arterial blood gas analysis at baseline in all children with rickets. Corrected serum calcium, then should be calculated using the formula:

Corrected Calcium = measured Calcium + 0.8 X (4 - serum Albumin).

Absolute value of creatinine may be misleading in children; hence eGFR should be calculated using the Schwartz formula to rule-out chronic kidney disease.

In patients with metabolic acidosis, the next step is measurement of serum AG [$AG = Na^+ - (Cl^- + HCO_3^-)$] and then calculation of albumin corrected AG using the formula: corrected AG = calculated AG + 2.4 X (4 - serum albumin). Wide reference ranges of 3.0 - 12 mmol/l to 8.5 - 15 mmol/l for the AG have been reported owing to difference in laboratory methods[11]. The authors use a reference range of

12 ± 4 mmol/l; however, clinician, should use their laboratory specific reference ranges.

Gastrointestinal (GI) loss of HCO_3^- due to diarrhoea, external pancreatic/small bowel drainage, ureterosigmoidostomy, jejunal loop and drugs like calcium chloride, magnesium sulphate, and cholestyramine simulate RTA due to presence of hyperchloremic normal AG metabolic acidosis. Urinary AG (UAG) measurement is the next step; GI loss of HCO_3^- is associated with negative UAG, while positive UAG suggests RTA[12]. UAG is calculated by the formula: UAG = Urine [($Na^+ + K^+$) – Cl^-]. A true AG, however, does not exist *in vivo* (serum or urine), since the sum of positive and negative ion charges must be equal. For an example, in urine, ($Na^+ + K^+ + NH_4^+ +$ unmeasured cations) = (Cl^- + unmeasured anions).

The difference between urinary unmeasured anions (sulfates, phosphates, organic anions) and unmeasured cations (calcium, magnesium) is relatively constant at an approximate value of 80, therefore urinary $Na^+ + K^+ + NH_4^+ = Cl^- + 80$, or $NH_4^+ = 80 – UAG$, or $UAG = 80 – NH_4^+$[13]. The equation, that is utilised to have an estimate of urinary NH_4^+ excretion, and numerically not much different from the above formula is urinary $NH_4^+ = 82 " 0.8 \times UAG$[14]. Positive UAG suggests more unmeasured anions (SO_4^{2-}, PO_4^{3-}) and minimal or no NH_4^+ likely due to RTA, while a negative UAG suggests adequate urinary NH_4^+ due to preserved urinary acidification system, hence GI loss of HCO_3^-. In summary, positive UAG (~ +20 to +90) in a background of normal AG metabolic acidosis is encountered in dRTA and pRTA when serum HCO_3^- is below threshold (14 - 18 mmol/l). On the other hand, a negative UAG (~ -20 to -50) suggests GI loss of HCO_3^- or pRTA with HCO_3^- above threshold (14 - 18 mmol/l).

However, there are certain limitations to the use of UAG[15,16,17,18].

1. UAG is of limited use if value falls between -20 and +20.

2. UAG is unreliable when urine pH exceeds 6.5. Urine pH of more than 6.5 suggests significant urinary HCO_3^-, an anion that is not taken into consideration while calculating UAG.

3. When anions other than $Cl^"$, such as β-hydroxybutyrate or acetoacetate in ketoacidosis, hippurate in toluene intoxication, acetylsalicylic acid, D-lactic acid and large quantities of penicillin are excreted in the company of NH_4^+, the value for NH_4^+ derived using the UAG will significantly underestimate the actual urinary NH_4^+ excretion. However, all these conditions are associated with high AG metabolic acidosis, and should not be confounding UAG in RTA. Increased unmeasured urinary cations like lithium may also interfere with UAG interpretation at times.

4. Acidification of urine requires adequate distal delivery of sodium. So, when distal Na^+ delivery is impaired, as suggested by urinary Na^+ < 20 - 25 mmol/l, usefulness of UAG is questionable.

In these above situations urine osmolar gap (UOG) is an effective alternative:

UOG = measured U_{osm} - calculated U_{osm}. Calculated U_{osm} = 2 X (serum [$Na^+ + K^+$] in mmol/l) + [blood urea nitrogen (in mg/dl)]/2.8 + [glucose (in mg/dl)]/18.

Modified UOG or UOG/2 is likely a true estimate of urinary NH_4^+, as it reflects the contribution of the anions accompanying NH_4^+ to the osmolality[19]. Urinary NH_4^+ of ≥ 75 mmol/l suggests intact NH_4^+ secretion, while urinary NH_4^+ of ≤ 25 mmol/l points towards inappropriately low NH_4^+ secretion. Some authors have suggested that UOG less than 40 mmol/l in patients with normal AG metabolic acidosis indicates impaired urinary NH_4^+ excretion, while urinary NH_4^+ is considered appropriately increased if the gap is above 100[11,13]. To summarise, UOG of less than 40 - 50 mmol/l in a background of normal AG metabolic suggests dRTA and UOG of more than 100 - 150 mmol/l points against dRTA.

Once the diagnosis of RTA is established, the next step is to identify its type. Freshly voided early morning, uninfected (urea spitting organisms are associated with falsely high urine pH) urine sample is tested for urine pH, a marker of urinary free H^+ concentration, preferably with a pH meter. Urine should ideally be collected under mineral oil to prevent dissipation of CO_2 and falsely elevated urine pH. Minimum achievable urine pH with normal renal function and acidification is 4.5 - 5.3. Urine pH > 5.5 in the presence of metabolic acidosis can be due to dRTA or pRTA with serum HCO_3^- above threshold or pRTA being treated with alkali. A filtered HCO_3^- that exceeds PCT reabsorptive capacity shall give falsely high urine pH. Urine pH < 5.5 during metabolic acidosis suggests pRTA with serum HCO_3^- below threshold. Metabolic acidosis and hypokalaemia associated with diarrhoea may increase renal NH_3 synthesis. In presence of normal distal tubular H^+ secretion, more renal NH_4^+ is produced, hence urine pH becomes alkaline (> 5.5) in diarrhoea. So, urine pH should always be performed once GI loss of HCO_3^- is ruled-out with negative UAG or high UOG. Urinary Na^+ less than 20 - 25 mmol/l is associated with low distal tubular H^+ secretion, hence, falsely high urine pH. A suggested approach to normal AG metabolic acidosis has been summarised in Fig. 4.

Other tests, that are used to assess distal acidification defects in patients of incomplete dRTA are ammonium chloride (NH_4Cl) challenge test, calcium chloride challenge test, frusemide plus fludrocortisone test and measurement of pCO_2 difference between urine and blood after $NaHCO_3$
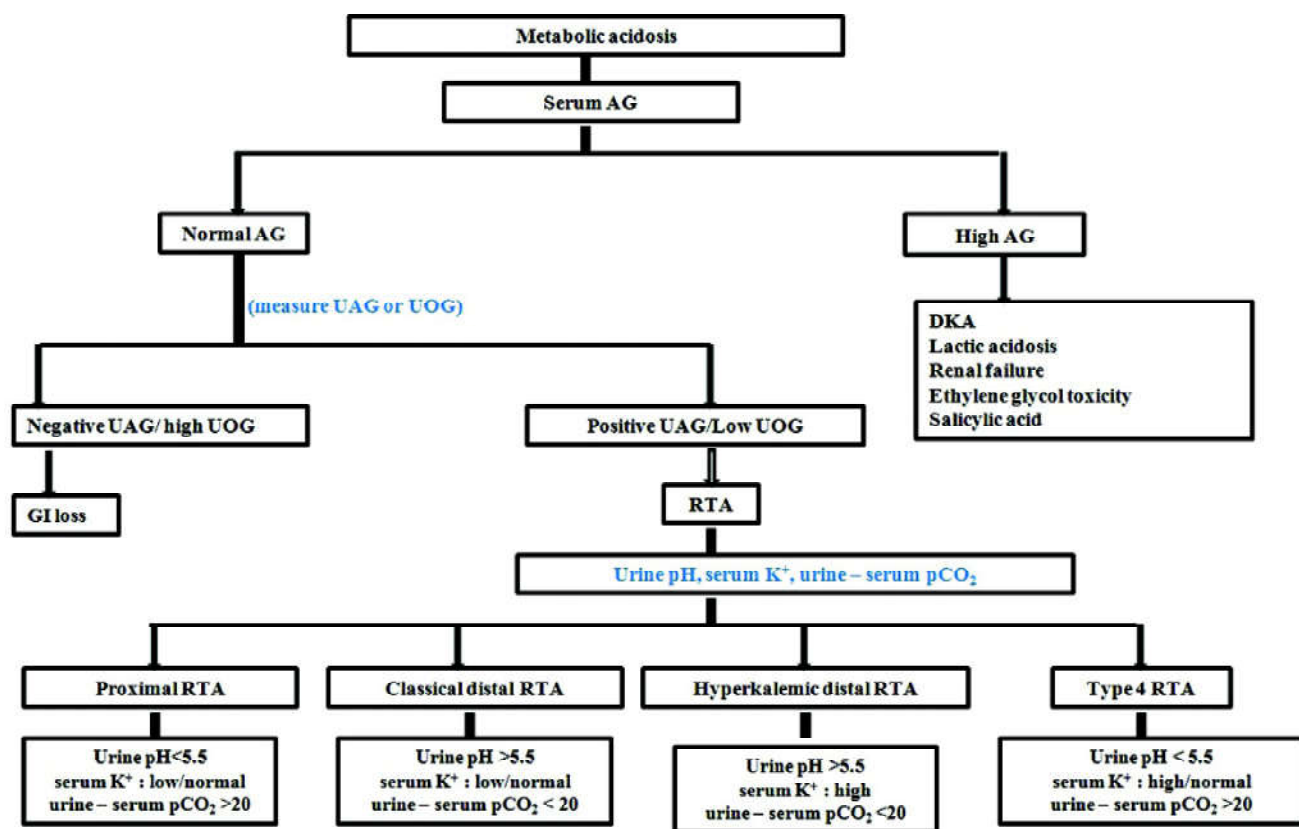
Fig. 4: *Approach to metabolic acidosis.*

infusion[20,21]. However, these tests are not required in a child with rickets secondary to RTA, as metabolic acidosis is florid in such cases.

pRTA is recognised by requirements for large quantities of base to raise serum HCO_3^- and appearance of bicarbonaturia at a normal serum HCO_3^- concentration. pRTA in steady state is associated with metabolic acidosis (HCO_3^-: 14 - 18 mmol/l), acidic urine pH (< 5.5) and low fractional HCO_3^- excretion (Fe-HCO_3). Fe-HCO_3 of more than 15 - 20% and urine pH higher than 7.5, when serum HCO_3^- is raised to normal values following infusion of $NaHCO_3$ confirms pRTA[22].

Type 3 RTA was formerly thought to be more widespread, when first identified. Infants with dRTA were routinely found to possess co-existing significant urinary HCO_3^- wasting. It is now acknowledged that most young children with dRTA experience an initial transient phase of bicarbonaturia as part of the syndrome's natural history. The precise mechanism(s) of proximal tubular dysfunction in dRTA is yet to be crystallised and two potential explanations have been put forward. Intracellular acidosis secondary to systemic acidosis induces endosomal dysfunction in proximal tubular cells in dRTA and results in proximal renal tubular cell dysfunction. Chronic hypokalaemia also induces a number of pathological changes in renal proximal tubular cells (infiltration with inflammatory mononuclear cells, vacuolisation, atrophy, destruction, brush border damage or even interstitial fibrosis) that culminates into proximal tubular dysfunction.

Unlike other forms of rickets, hypophosphataemia is uncommon in rickets associated with RTA. In a patient of hypophosphataemia, renal loss of phosphate should be differentiated from non renal cause of phosphate wasting by calculating tubular reabsorption of phosphate (TRP) and tubular maximum for phosphate corrected for GFR (TmP/GFR). Phosphate reabsorption occurs mainly in the PCT, which reclaim roughly 80 - 85% of the filtered load. Additional 8 - 10% phosphate is reabsorbed in the distal tubule (but not in loop of Henle), leaving about 10 - 12% for excretion in the urine. The normal TRP, therefore, is about 90%[23]. TRP is calculated using the formula 1-[(Up/Sp) X (Scr/Ucr)] (U: urine; S: serum; p: phosphate; cr: creatinine).

TmP/GFR is maximum renal tubular phosphate reabsorption in mass per unit volume of glomerular filtrate. It is independent of the rate of phosphate flow into the extracellular space from gut, cells and bone and glomerular filtration rate[24]. It was initially developed to differentiate hypercalcaemia due to hyperparathyroidism from other causes of hypercalcaemia that is now done by measuring

PTH levels[25]. If TRP is less than or equal to 0·86 then TmP/GFR can be derived from standardised nomogram or multiplying TRP by serum phosphate. If TRP is greater than 0·86, Kenny and Glen's equation is used ['x' = (0·3 X TRP)/{1-(0.8 X TRP)}] and TmP/GFR= 'x' X serum phosphate][26,27]. TmP/GFR is compared with age and sex specific range and normal value roughly corresponds with age and specific reference range for plasma phosphate. Low TmP/GFR in presence of hypophosphatemia suggests renal phosphate loss[28]. Hypophosphatemia in RTA is secondary to renal loss, and likely due to pRTA. The affected child often has co-existent glycosuria, aminoaciduria, low-molecular weight proteinuria, hypercalciuria, uricosuria in varying combinations as a part of Fanconi syndrome. However, as discussed earlier, primary dRTA is also associated with reversible form of generalised defects in proximal tubular absorptive capacity resulting in phosphaturia, low molecular proteinuria, but, not glycosuria. Moreover primary hypophosphatemic rickets or calciopenic rickets, by virtue of severe hypophosphatemia, may result in impaired HCO_3^- reabsorption from PCT (pRTA) or acquired, reversible distal acidification defect (dRTA). In addition to hypophosphatemia, secondary hyperparathyroidism associated with rickets associated with abnormal vitamin D homeostasis, also contribute to pRTA as PTH inhibits proximal tubular bicarbonate reabsorption by interfering with the activities of apical Na^+/H^+ exchanger (NHE3) and the basolateral Na^+/K^+-ATPase. Clinicians need to be vigilant to identify the underlying primary aetiology in children with rickets, normal AG metabolic acidosis and hypophosphatemia.

Once the type of RTA is identified in a child with rickets, next step is to rule out important secondary causes and mutational analysis for genes responsible for primary forms of RTA (Table I). At times, certain clinical clues may help to target specific genes for analysis. Accompanying features of CA-II mutation has already been discussed. In addition, eye changes and basal ganglion calcification in pRTA suggests NBC-1 defect, sensori-neural deafness in dRTA points towards H^+ ATPase abnormality, haemolysis with dRTA suggests defective AE1 (Table I). pRTA combined with epilepsy and osteopetrosis suggests involvement of the renal chloride channel (CLCN) gene 7 (CLCN7). Dent's disease, an X-linked condition due to defective renal CLCN5, is associated with vitamin A-responsive night blindness, hypophosphatemic rickets and generalised PCT dysfunction, and closely mimics pRTA[29]. Recently, a second variant of Dent's disease (Dent 2) due to mutation of oculocerebrorenal syndrome of Lowe gene 1(OCRL1) has been identified[30].

Treatment

Alkali replacement is the mainstay of therapy in all forms of RTA with rickets. 1 - 1.5 mEq/Kg of non-volatile acids are generated normally per day that is excreted in the form of

Fig. 5: *4.5-year-old girl with dRTA was treated with alkali therapy. Note the complete clinical (B) and radiological (D) recovery after 1.5 years of treatment. A and C represent features at presentation.*

Fig. 6: *Residual deformity after 3 years of alkali therapy in a boy diagnosed with dRTA at 16 years of age. The growth plates are fused and the boy is posted for corrective osteotomy.*

titrable acids/NH_4^+). Daily alkali requirement in RTA should take into account the H^+ retained each day and urinary bicarbonate loss, which however is negligible in dRTA. The usual daily dose of alkali in dRTA is 1 - 2 mmol/Kg in adults and 4 - 8 mmol/Kg in children. Rapidly growing skeleton generates additional acid load in children. In addition, higher fixed urine pH in children is associated with relatively larger urinary bicarbonate loss compared to adults. Sodium bicarbonate or sodium citrate is often used and titrated to achieve and maintain normal serum HCO_3^- (22 - 24 mmol/l). Correction of acidosis reduces urinary K^+ and prevents hypokalemia, and patients may not require potassium supplementation in the long run. However, in presence of hypokalemia, potassium citrate is preferred.

In contrast, owing to marked urinary HCO_3^- loss in pRTA, daily alkali requirement is much higher, 10 - 30 mmol/Kg, along with large supplementation of K^+. Increased distal tubular Na^+ and HCO_3^- delivery stimulates K^+ secretion, hence, potassium citrate with/without sodium bicarbonate is the preferred form of therapy. Near normal HCO_3^- in children needs to be achieved. If large dose of alkali is ineffective to achieve target HCO_3^- or such a high dose is not tolerated, thiazide diuretics may be added. Mild volume depletion associated with thiazide diuretics enhances Na^+ and HCO_3^- absorption in PCT. Those with severe hypophosphatemia should be co-prescribed phosphate supplement and active vitamin D metabolites.

Conclusions

RTA is a complex disease and, at times, difficult to diagnose due to the variable presentation. RTA is known to be associated with rickets, and RTA needs to be ruled-out in all cases of 'refractory rickets'. Arterial blood gas analysis is recommended at baseline in children with rickets along with other first-line investigations. Evaluation for RTA begins with measuring serum AG in individuals having metabolic acidosis. Patients with normal AG metabolic acidosis should undergo testing for UAG with/without UOG. Once the cause is established to be due to RTA, urine pH can guide for confirming the specific type of RTA. Early recognition and specific management is rewarding as it enables relief of symptoms and complete clinical and radiological remission (Fig. 5). If diagnosed late, deformity might be permanent, once growth plates are fused, that ultimately require corrective osteotomy.

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Are Artificial Sweeteners Safe and Useful: What does the Evidence Suggest ?

Rudrajit Paul*, Dipanjan Bandyopadhyay**

Introduction

Obesity and diabetes are major non-communicable diseases of modern times. As people are becoming aware of the dangers of metabolic syndrome, they often try to cut down on their carbohydrate or sugar intake. In this background, there is a rising public interest in so-called "zero calorie" artificial sweeteners. Artificial sweeteners are used in various food items available in the market and many manufacturers also use a blend of sugar and artificial sweeteners. The global market for artificial sweeteners is expected to grow from $ 7.2 Bn in 2018 to $ 9.7 Bn in 2024[1]. The soft drink industry is expected to have the maximum consumption of this ingredient due to demand for low calorie drinks or "diet" drinks[2]. Asia-Pacific region is the largest market for this food additive and is expected to grow quickly in the coming years[2].

In India, sweets are an integral part of everyday meals for most communities. Indians are the largest consumers of sugar in the world[3]. However, with the rising prevalence of diabetes and obesity, many Indians are becoming aware of the dangers of refined carbohydrates. In such a scenario, the demand for artificial sweeteners is rising rapidly[3]. The current market for this ingredient is estimated at 150 crores and is expected to have a double digit growth in the coming years[3]. For example, Saccharin, a commonly used sweetener in India, had a market volume of more than 4,600 tons in 2018 and is expected to reach 5,300 tons by 2024[4]. Since sweet dishes are an integral part of all religious rituals and family programmes in India, the demand for sweeteners will always be there. This is different from Western societies, where sweetened beverages and candy form the main route of intake of sugar.

In 1976, two researchers were working at Queen Elizabeth College, London with chlorinated sugar compounds. One of them, Leslie Hough, asked the other, Sashikant Phadnis, to test a compound. But Phadnis thought that he had been asked to "taste" it. So, he tasted the compound, called sucralose, and found it to be very sweet. Thus, sucralose was discovered accidentally.

Sweet taste is perceived by taste receptors expressed on taste cells. There are many types of taste receptors. For sweetness, it is T1R2 and T1R3. In T1R subunit, there is an extracellular domain called venus flytrap (VFT). Sucralose and natural sugars like sucrose or glucose bind to VFT domains of both T1R2 and T1R3 while Aspartame binds to only T1R2.

These artificial sweeteners or non-nutritive sweeteners (thus labelled by the AHA) are marketed directly in India and do not require any authorisation by health professionals. Thus, consumers are able to use them at their will, based on the advertisements and hearsay. But the consumers are often not conversant with the recommended daily intake limit or similar other scientific facts. This lack of awareness can cause excess consumption and related side-effects. For example, as people are becoming aware of the large amount of sugar added to popular carbonated beverages, many are thinking of switching to "diet" versions of those drinks. But these artificially sweetened beverages may not help in cutting down body weight at all. The San Antonio heart study in 2008 found that regular intake of artificially sweetened beverages led to an increased risk for obesity. But these facts are often suppressed during aggressive marketing.

The various online marketplaces have a wide variety of these sweeteners and consumers can decide for themselves. These sweeteners are marketed as a panacea for obese persons and celebrities often endorse them as means of staying healthy. However, the health effects of these sweeteners are a less discussed topic and consumers taking excess dose of these chemicals may present to the physician with side-effects. Thus, physicians should be aware of the biochemistry and pharmacology of these artificial sweeteners.

Common sweeteners used

Various artificial sweeteners are available in the market. The common ones are Acesulfame K, Aspartame, Neotame, Sucralose, Saccharin, Alitame and Cyclamate[5]. These sugar substitutes add sweet taste to food without adding the extra calories of sugar. Usually, these sweeteners have much

*Associate Professor, Department of Critical Care Medicine, IPGMER and SSKM Hospital, Kolkata - 700 020; **Associate Professor, Department of General Medicine, North Bengal Medical College, West Bengal.
Corresponding Author: Rudrajit Paul, Associate Professor, Department of Critical Care Medicine, IPGMER & SSKM Hospital, Kolkata - 700 020. Phone: 9433824341; E-mail: r.paul.medicalcollege@gmail.com.

more sweetness compared to sucrose (for example, sweetness of Aspartame is 200 times sucrose and that of Saccharin is 300 times that of sucrose)[5]. Thus, they are needed in much smaller amount in food compared to sucrose. Sodium cyclamate is a compound with sweetness 30 - 50 times that of table sugar (Sucrose), thereby making it the least potent of all available artificial sweeteners. It is banned in the USA for fear of carcinogenicity, although it is approved in many other countries.

Aspartame is a dipeptide. It is heat labile and thus cannot be used for baking or cooking[5]. Right from the time of its approval, aspartame has been the subject of debate and public scrutiny. At one time, aspartame was rumoured to be carcinogenic. But subsequent human studies did not find any evidence to support this claim[5].

As of June 2019, the following sweeteners are approved in India (by FSSAI): Saccharin, Acesulfame, Aspartame, neotame, sucralose, and isomaltulose[6]. (However, it must be remembered that in the current age of frequent international travel, these countrywise approvals really do not matter. Anyone can go to USA or Europe and consume food products with artificial sweeteners. Then, they can come back to India with adverse health effects and present to the physician here. In India, these sweeteners are mixed in a variety of food items like chocolate, Pan masala, carbonated beverages, sweets, and chewing gum[6]. Aspartame has an even wider scope of application with usage in icecream, yogurt, flavoured milk, and jam, in addition to those mentioned above[6]. Sucralose (since it is stable at high temperature) is further used in cookies, pastry, doughnut, and custard powder[6]. Thus, for all varieties of sweet dishes and other food items where sweet additives are required, suitable artificial sweeteners are available.

In India, one important consideration for the use of non-nutritive sweeteners is during cooking. Daily Indian meals involve curries and in all parts of India, preparation of many curries requires sugar in addition to various spices. But all of these compounds are not heat stable. Thus, for use in cooking, only heat stable ones like acesulfame and Sucralose are to be used[6]. One disadvantage of acesulfame is that it sometimes leaves a bitter after-taste[5]. Thus, acesulfame may have to be combined with sucralose to avoid this bitter after-taste. This is especially true if acesulfame is used in alcoholic beverages[5]. Neotame is approved for use only in soft drinks and isomaltulose is approved only for sweet confectionary[6].

Most of these artificial sweeteners in food are not absorbed from the GI tract and are excreted in the faeces. Only those which are peptide by chemical composition like aspartame and alitame, are absorbed and metabolised by the amino-acid pathway.

In the USA, the USFDA has approved six sweeteners[7]: saccharin, aspartame, acesulfame potassium (Ace-K), sucralose, neotame, and advantame. Advantame is a compound which is 20,000 times sweeter than sucrose. It was approved in 2014. It is heat stable and can be used for baking. However, it is not recommended for use in poultry items.

Xylitol:

This is a 5-carbon compound which is sometimes used as a sweetener. It is synthesized from lignocellulosic biomass like hardwood, softwood, or agricultural wastes of maize or wheat. It is not a zero-calorie sweetener like the other compounds mentioned in this article, but calorie count is much less compared to sucrose. One particular effect of xylitol is reduction of *streptococcus mutans* in oral flora, thereby reducing the risk of dental caries.

Stevia:

Recently, a plant derived artificial sweetener, called Stevia has become available in the market. Stevia plant, native to South America, is a member of the sunflower family[8]. Knowledge about sweetness of various parts of this plant was known to the indigenous tribes of Brazil and Paraguay for centuries. Commercially, the ingredient is extracted from the leaves of the plant. The sweet ingredient is Steviol glycosides like Rebaudioside (A to F) and Dulcoside A[8]. This ingredient is used in a variety of food items like dairy products, ice cream, or canned fruit[7]. It is heat stable and thus, can be used for cooking or baking. FSSAI approved Stevia for the Indian market in 2015. But acceptance of stevia by Indians is slow. However, recently, some soft drink manufacturers in India have released stevia-based versions of their drinks and the market is slowly expected to grow.

FDA opinion on Stevia[9]

In the FDA website, as of 2018, it is written that high purity steviol glycosides are generally considered as safe (GRAS) and do not need FDA approval for use in food. But stevia leaves or crude extracts are not approved for use.

However in India, various online stores sell Stevia leaf also. Any reader of this article can now visit any online store and buy stevia leaves at his/her will. Whether use of these leaves is advisable and if so, how they should be used, is a matter of debate, but there is no regulation on their retail.

Recent interest in "rare sugars"[5]

Recently, there is considerable interest in the field of dietetics in rare sugar compounds. These are monosaccharides which are not metabolised in the body.

They can act as food sweeteners and one advantage, compared to other artificial sweeteners, is that there is no bitter after-taste.

Some examples include Xylitol, Tagatose and D-psicose. Readers are encouraged to read the relevant literature on their properties for further information.

Possible health effects

Low or zero calorie sweeteners are expected to reduce the intake of carbohydrates without compromising the taste of food. Reduced intake of carbohydrates is expected to reduce body weight. *But is the cause-effect relationship so simple?*

In 2012, a landmark meta-analysis on the effect of sugar intake on body weight was published in the BMJ[10]. This meta-analysis, commissioned by the WHO, tried to find out the quantitative effect of excess sugar intake on body weight, based on published trial data. However, most of the trials included in this meta-analysis had very small number of participants. For example, the trial by Markmann *et al* in 2000 included only 20 subjects and that by Poppitt in 2002 included 28 subjects[10]. Thus, the results from these trials may not be generalised. Moreover, many of these trials showed that increase in sugar intake led to increase in waist circumference but the increase in actual body weight was modest[10]. For example, the 2011 Nurses' Health study I and II in USA found that increase in sugar intake by 1 serving/day led to increase in body weight of around 0.5 kg over a 4-year period[11]. For the present article, the significance of these data lies in the fact that replacement of sugar with artificial sweeteners will not lead to a radical decrease in body weight. If the total calorie intake remains same, then only artificial sweeteners *will not help in reduction of obesity*.

Now the question arises, how much amount of these artificial (or high-intensity) sweeteners is safe? For this, the FDA uses a parameter called ADI: Acceptable daily Intake. This is the amount of substance which is considered safe for daily consumption over the course of lifetime of a person. For example, ADI of saccharin is up to 5 mg/kg and for Stevia it is 4 mg/kg. Thus, Stevia has a narrow window of use and Stevia-based drinks cannot be taken in large quantities. But the problem is there is no authoritative mechanism for limiting the sale of these items and a person may consume as much as he/she wants at a time. The consumer product companies would just write a statutory warning in the label and thus, wash their hands off. This raises the possibility of side-effects from excess use.

Another problem is that the ADI for each sweetener compound may vary according to the recommending authority. For example, as stated above, the ADI for saccharin is 5 mg/kg according to the European Food safety Authority but according to the Joint Food and Agriculture Organization of the United Nations, the figure is 15 mg/kg[12]. For sucralose (perhaps the commonest sweetener used in India) the Joint Food and Agriculture Organization of the United Nations limit is 5 mg/kg; but European limit is 15 mg/kg.

Also, organisations like the FSSAI have demarcated maximum permissible concentration of sweeteners in each food item[6]. If these guidelines are followed strictly, then the chance of adverse reactions is very low. For example, for carbonated beverages, the limit of saccharin is 100 ppm and for chocolates, it is 500 ppm[6]. For aspartame, the limit for custard powder is 1,000 ppm and for chocolate, it is 2,000 ppm. Similar guidelines may be found for each sweetener and for each food item.

In 2019, in the BMJ, another important meta-analysis was published. This one analysed the link between intake of non-sugar sweeteners and health outcomes[12]. The trials included in this meta-analysis were mostly small and there were some lacunae on the reporting of data. Analysis revealed that the reduction in body weight after use of non-sugar sweeteners (NSS) was modest at best[12]. For obese individuals, the mean reduction in body weight was 2 kg (pooled data of 3 studies). But many trials found no significant weight reduction after the use of NSS. So, at this time, NSS cannot be recommended for control of obesity.

It is expected that diabetic patients using NSS should have lower blood glucose levels as they are replacing sugar with a no-calorie substitute. But in practice, data shows that the reduction in fasting blood glucose is modest at best for persons using NSS[12]. Published data also show that the use of aspartame in diabetics may act as a chemical stressor and lead to increased cortisol levels and thus, increase in blood glucose levels[13]. It may also alter gut microbial activity and increase insulin resistance paradoxically. Recently (2017) a European study revealed that chronic consumption of NSS actually led to increased risk of type 2 diabetes, when adjusted for BMI[14]. In this study, it was also shown that NSS conferred an increased risk of diabetes in a dose-dependent manner. The use of NSS may be associated with paradoxical weight gain due to increase in appetite and thus, increased calorie consumption as a whole[15]. *So, the simple explanation that replacing sugar with NSS will control blood sugar levels is not true*.

In 2012, the American Heart Association also published a statement on NSS[16]. In this, the association concluded that there is still insufficient evidence to recommend NSS as a health supplement[16]. Also, they made some interesting observations. It was recorded that the use of NSS did not

have any clinically meaningful effect on glycaemic control in diabetics[16]. The association of "diet" soft drinks or artificially sweetened beverages (ASB) with cardiovascular events is debatable. While some studies have found a statistical association of ASB with coronary events and chronic kidney disease, other studies have reported no association. One problem with these studies is that most American and European studies have reported data on NSS used in beverages. But in India, most sugar intake is in form of sweets or dairy products. Thus, whether the metabolic effect of NSS in these products will be similar to ASB is a matter of research. Also, whether NSS added to cooking will have the intended health effects is a matter of speculation.

Molecular effect of NSS in the brain

A 2016 study from Sydney found that chronic sucralose diet in animals paradoxically increases food intake[17]. Thus, although the intake of sugar is reduced, the organism can end-up eating more as a whole, thereby increasing calorie intake[17]. Some changes in the pleasure-reward pathways in the brain is said to be responsible for this effect. The applicability of this data in humans is still debatable. However, some molecular mechanisms have been proposed which may explain this paradoxical increase in energy intake after taking NSS[16]. One such theory is that, intake of sugar leads to release of gut peptides which causes satiety in the hypothalamus. But intake of NSS does not release gut peptides and thus, the person ends up eating more.

Other health hazards

Another health hazard often cited of NSS is the risk of carcinogenicity[15]. Cyclamate was the first compound to be implicated in causing cancer, and thus banned in the USA[15]. Although later studies refuted this claim, still the compound remains banned in the USA. For other sweeteners, no link has been found with cancers[15]. In the 1970s, saccharin was linked to bladder cancer in rats. But subsequent human studies did not find any risk[18]. Now-a-days, a number of artificial sweeteners are combined in the same food item (as stated above for Acesulfame). Thus, individual effect of one compound is difficult to gauge.

Aspartame is metabolised to phenylalanine. Thus, patients with phenylketonuria should never be given this compound[15]. Aspartame, in general, can also cause chronic fatigue[15].

There is some data on the potential for excitotoxicity of Aspartame and Neotame. But whether this is clinically meaningful at the usual level of intake is doubtful[19].

Consumers in many countries have concerns about the health safety of NSS[16]. In a survey conducted in the USA, 64% of the subjects indicated that they were concerned about the safety of these sweeteners[16]. Thus, manufacturers often use certain tricks of wordplay to bypass this anxiety. For example, Stevia is marketed as "natural" sweetener, obtained from leaves of certain plants. Sucralose is marketed as "made from natural sugar" as it is a chlorinated form of sucrose.

Physicians working in India will often come across patients taking a lot of herbal supplements as medicines. Many of them have the notion that honey or jaggery is allowable in diabetic patients, instead of sugar. But this myth should be dispelled. Both honey and jaggery contain sucrose, and thus the net effect on calorie intake is the same. They are not sugar substitutes. Chawanprash is another ayurvedic concoction widely used in India. Recently, consumer companies have launched "sugar-free" chawanprash. These contain the artificial sweeteners discussed above. Consumers should be made aware of this change.

Conclusion (Take-home messages)

- Artificial sweeteners available in the market are generally safe for consumption, with certain restrictions.

- Artificial sweeteners are not a solution for diabetes control or obesity.

- There is a chance in alteration of appetite or feeding behaviour of a person taking artificial sweeteners.

- In India, heat-stable sweeteners like sucralose can be used for cooking but their health effect is still not known.

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Role of Posters in Enhancing HIV/AIDS Awareness

Atul Kakar*, Richa Dewan**, Shikhar Tripathi***, Atul Gogia**, Nakul Gupta****

Introduction

India has the third largest HIV epidemic in the world, with approximately 2.1 million people living with HIV/AIDS (that is 0.2% prevalence) and 0.1% incidence, over last year[1]. The 90 - 90 - 90 target set by the United Nations has been envisioned by India, that by end of 2020, 90% of people living with HIV (PLHIV) will know their HIV status, 90% of people who know their HIV-positive status will be accessing treatment and 90% of people on treatment will have suppressed viral loads[2]. Achieving this is still a challenge for India, inspite of the awareness, surveillance, free drug distribution by National AIDS Control Organisation of India. There is an urgent need for health education to reduce incidence of HIV/AIDS in the future. Posters are one of the important instruments for health education amongst general population. This review will examine the utility of posters for spreading awareness about the various issues related to HIV/AIDS and the quality of life of PLHIV based on the currently available literature.

Manna of posters

Poster presentation falls under the category of small media and is an effective mode of propaganda communication which involves both intellect and creativity. Posters provide an effective mode of delivering a message and are the commonest and most rapid way to disseminate information[3]. They are also the easiest and the most cost effective way to spread a word. A simple, small media poster has the power to become a multimedia trend if it strikes the right cord with a viewer, who then might share the poster as a photograph and forward it to his/her peers, and the chain would go on.

Qualities of a good poster

A poster is supposed to catch the attention, inform, convince and provoke. If the viewer feels addressed, a poster can influence his or her decisions through text and images. An optimally designed poster appeals to the viewer's curiosity and intellect. A good quality poster must attract attention, be memorable and have an interesting design with a clearly structured message or statement. Posters are known to effectively stimulate the psychomotor skill of learning in a viewer. They act as a medium of communication which serves to elicit attention and focus on the centre of interest. They are effective if the images and slogans can be identified easily (Fig. 1). The appeal of the message allows the public to accept it on a widespread spectra of emotional, social, and intellectual levels. The poster should be in a language which is understood by the target group. It is not necessary to use texts, the language of illustrations is equally potent in delivering a desired message[4].

Efficacy and shortcomings of posters

Posters in public spaces (pillars, parking lots, restrooms, hospital waiting areas, metro/bus stations, outside cinema multiplexes, etc.) are subject to numerous external influences such as the type of environment, lighting conditions, weather conditions, competition with other close by visual media, and partial concealment by persons or objects. The sum of these influences, and not always the optimal framework conditions, have a great impact on the visibility and readability of posters. All these factors, clubbed together, impact the efficacy of the poster.

A significant shortcoming in any poster can be inclusion of too much information (Fig. 2). Rege concluded in his study that for a poster to be good, the message should be so simple and short that the explaining and remembering become synonymous[5].

HIV: The issue at hand

Inspite of low prevalence, India still has a large PLHIV population, and the matter of concern is new infections and HIV related deaths[1]. The current incidence of HIV is much more potent than it's possible prevention. Therefore the posters can be utilised as a mass accessible media for spreading awareness among the general population. In a

*Vice Chairman and Senior Consultant, **Senior Consultant, ***Oberver, ****Consultant, Department of Medicine, Sir Ganga Ram Hospital, Rajendra Nagar, New Delhi - 110 060.
Corresponding Author: Dr Atul Kakar, Vice Chairman and Senior Consultant, Department of Medicine, Sir Ganga Ram Hospital, Rajendra Nagar, New Delhi - 110 060. Phone: 9811110802, E-mail: atul.kakar@sgrh.edu.in.

cross-sectional study it was reported that 67.9% of the subjects had received their very first piece of knowledge about HIV/AIDS via posters[6].

Fig. 1:
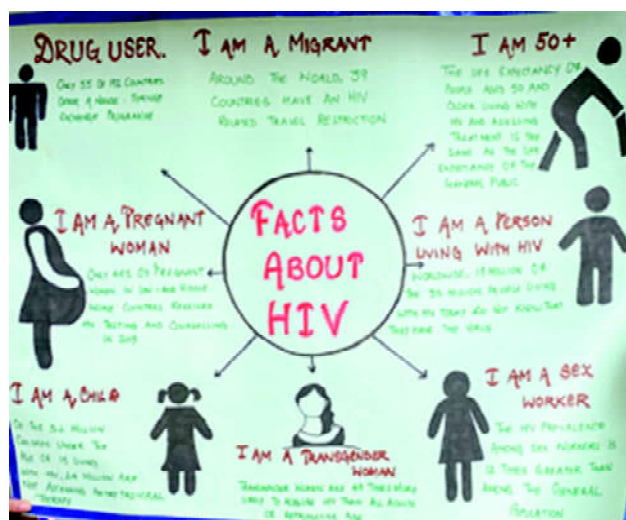
Fig. 2:

These posters can be intended for a specific target group, i.e., people who are already infected with HIV or for others at risk of acquiring new infection by way of high risk behaviour. It is essential to educate people about the modes of transmission of HIV and how it can be prevented by the use of preventive measures like using condoms and not sharing needles amongst Intravenous Drug Users (IDUs) (Fig. 3). Also, the people who might have already been infected with HIV and are unaware of their condition need to be made aware of the onset of symptoms (Fig. 4) once the infection has taken place so that they can identify these

Fig. 3:

Fig. 4:

and then get themselves tested and become aware of their HIV status. In a study on pregnant mothers, it was found that 37% of the mothers received knowledge and awareness about possibility of mother-to-child transmission of HIV via posters and that prompted them to visit the antenatal clinic for their check-ups[7].

Posters can also be helpful in combating the social stigma against HIV/AIDS and informing PLHIV about social and legal welfare schemes (Fig. 5). Today, with the wide spectrum of effective antiretroviral therapies (ART) available, posters can be utilised to inform the public about availability of these therapies and the treatment clinics (Fig. 6).

The right impact

Posters may not give in-depth information, but they can give a 'food for thought' and incite curiosity in the viewer to take out time and read about that one particular sentence, or even a word, which they saw on the poster. In their study, Lubinga *et al* reported that actual comprehension (AC) was initially low upon first encounter with a deliberately puzzling poster on HIV/AIDS but after the surveyors gave the subjects some time to have a conversation with their
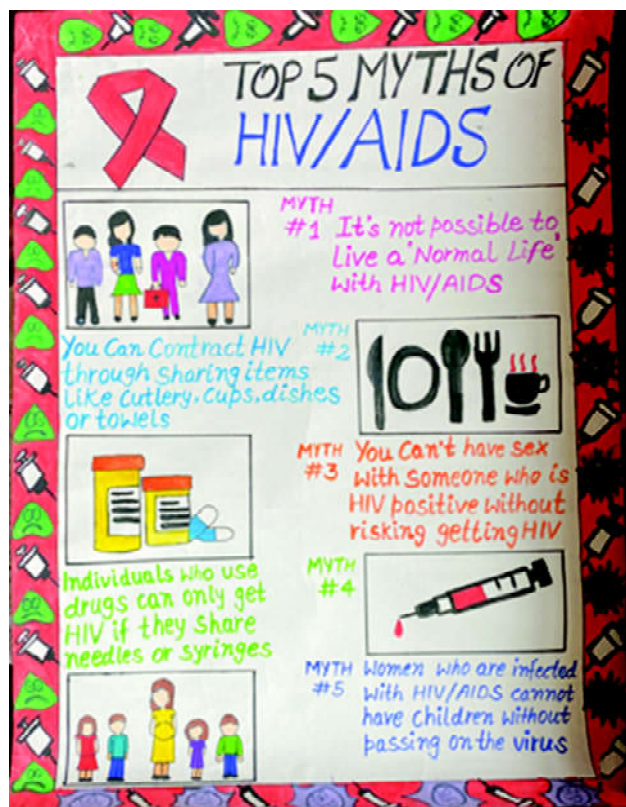
Fig. 1:
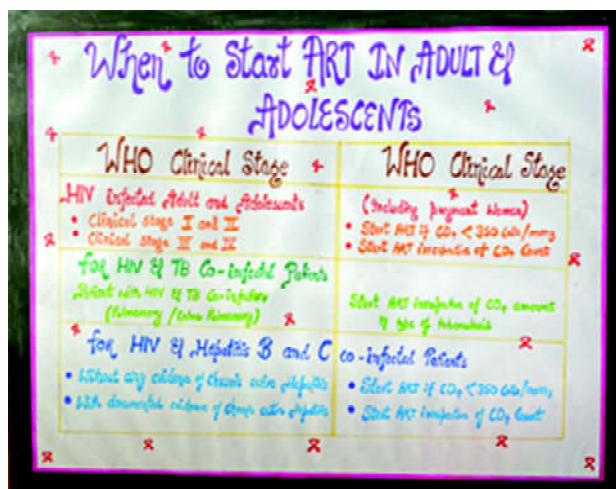
Fig. 6:

peer group about it and to further read about the message, it was seen that the actual comprehension was greater than the perceived comprehension[8].

This sort of curiosity and excitement cannot be induced by larger media since they have too much information to be looked upon and in today's fast moving life, no one has the time to ponder on that one word amongst a thousand that they might read in a book or in an information pamphlet, but one word out of the hundred in a poster can do this magic. Also, if present at a place where the viewer has to be stationary for a long-time (e.g., metro rail, offices, waiting areas of hospitals, or universities), then a poster can have a deep and promising impact on the viewer's mind. This was also studied by Ward *et al* on patients waiting in the reception area of their general practitioners, wherein they reported that 82% patients read the posters placed in the waiting area, and 95% of those remembered the information mentioned in the posters[9].

In a long duration study conducted in Mexico, Tepichin reported that out of the multiple methods used to convey the same information across the four phases of their study, posters were the most impactful medium. While other media also received certain backlash, posters were highly useful in disseminating knowledge related to HIV/AIDS, which was accepted by teenagers and older age groups alike[10].

Accessibility

Since its invention, internet has proven to be the most potent way of disseminating knowledge and information, but it is a known fact that almost 45% of the world

population and 60% of Indian population is still without internet and multimedia access[11]. In such a scenario, posters can act as an excellent medium of spreading awareness amongst all strata of the community equally. Xiao *et al* reported in their study based in China that exposure to HIV/AIDS prevention information delivered by posters, Internet and the other forms of media was found to be associated with communication about HIV or condom use with sexual partners and their study showed an increased trend of awareness regarding issues related to HIV/AIDS[12]. In India, posters should be put up at every possible display area ranging from those in villages to those inside MNC lounges in skyscrapers. Regarding HIV, posters can be prepared for such a wide array of dominions (awareness for prevention, modes of transmission, onset of symptoms, availability of treatment, government schemes for PLHIV, etc.) each of which will prove to be extremely helpful in creating a positive propaganda towards management of HIV/AIDS.

Preparing a poster

Preparing an effective poster involves a multistage process starting from planning and organisation, to picking the right information and the figures to be displayed, and to its presentation at a spot where it is accessible to a greater mass. The first step is planning for the poster, this includes deciding upon a topic that needs to be highlighted, the way in which it should be highlighted and the group that has to be targeted. The next step is organising the available data and illustrations into a meaningful and eye-catching poster. It is important to keep the font size big enough to be seen from some distance. It is also important to maintain a contrast relationship between the background and the font colour and to highlight the important words/phrases as much as possible. The main aim should be drawing attention towards the essence of the poster and the data upon which the poster should incite the viewer to further ponder upon.

Stage 1: Planning

The very first step of preparing a poster is planning about what is to be displayed. It is an integral step since it determines how impactful the message can be. It must involve deciding upon which aspect of information related to HIV is to be displayed and how it has to be displayed - via text or via illustrations. Another aspect of planning has to be the determination of a target group. To convey the same information, different methods can be required while targeting different groups in the society. A viewer's reaction to a poster depends on his interests, inclinations and especially on his social situation. Consequently, each viewer may interpret a poster differently, based on his/her origin, the background, and socio-financial realities. In a study conducted by Quek *et al*, it was reported that subjects from low-income families and relatively less academically successful schools knew significantly less than other subjects[13]. This highlights the importance our last point that different groups require different methods to be conveyed the same information. Simpler terms and easily recognisable illustrations should be used in posters aimed at propagating knowledge to aforementioned group of subjects.

Stage 2: Organisation

Organising the available data into a meaningful propaganda is an important part of the complete process of making a poster. It must include organising the more important data in a more highlighted way than the lesser important texts. As correctly pointed out by Laver, educating about prevention of HIV/AIDS is more than telling people what not to do[14]. In the step of organisation one must keep in mind to give optimum weightage to actions which must be done by people who have not contacted HIV (like condom use, regular check-ups, etc.) and the ones that must be done by people living with HIV (like undergoing antiretroviral therapies, informing previous partners about their condition, etc.).

Stage 3: Printing

In this step it is essential to make sure that the quality of the print as well as the paper is up to the mark. It should be kept in mind that a lot of pictures and illustration stand to pixelate upon printing on a larger sized paper, hence photographs and illustrations of higher resolutions should be preferred. If the message has to be delivered on a smaller scale and to limited audience, traditional chart paper and crayons can be used to prepare a poster. This will also be useful in scenarios where limited resources are available. Graphic aids like photographs, pictures, charts, graphs and cartoons can be used to convey longer messages in smaller space. This step must also include choosing the right colour combination between the background and the foreground of the poster so as to make sure that all the information is visible appropriately, even from a considerable distance.

The following considerations must be kept in mind:-

- Format/size: Generally, a large format is more noticeable, but a smaller, very bright and colour-intensive poster is more noticeable than a large, dark-coloured poster.

- Colour of the paper/background: Use light, not very cloudy colours. For darker colours, the contrast should be considered.

- Contrast: Strong contrasts (e.g., light-dark or complementary contrasts) are striking; however, an overload of contrasts should be avoided.

- Size and conspicuousness of the motif: Choice of an appealing motif in a size appropriate to the format, wherein originality and recognition should be the drivers.

- Title/slogan: Most effective and appealing slogan (an explanation, question or antithesis) should be used, so as to give out a much larger information in much lesser words.

- Type and size of the font: Choose a font that is easy to read and in line with the "character" of the poster.

- Remote readability: All the mentioned points have a major impact on the perception of the poster, thus the context of use must be considered.

- Contents/information: Information on the message to be conveyed should be legible and included in "exposed" areas of the poster.

Stage 4: Presenting the poster

AIDS posters are a material objects and the image of AIDS is considered in terms of their function and for a regime of power centred on the human body[15]. Their conclusion is suggestive of the importance of right presentation of a poster on AIDS which marks the most important step of making and presenting a poster. Not only the site but also the way of presenting a poster leaves a lasting mark inside the mind of a viewer.

Not much is required to be done if it is an audiovisual poster, but in a small media printed poster a lot of thought has to be given to the site where the poster must be placed. The determination of site must include the proposed target group as the criteria because placing a poster intended for the low-income group inside an expensive shopping complex would practically be a waste. Also, it must be kept

in mind that presentation must include components which would give a positive outlook to the poster and can virtually speak for the poster. For example, placing a poster on promotion of condom use near a pharmacy would encourage people more towards condom use than placing the same poster next to a cinema multiplex.

Topics to be covered

The topics for posters will be according to the priorities fixed by the healthcare providers and planners. In an exploratory study, it was found that inclusion of both gain- and loss- messages in posters lead to better impact on the viewer[16]. Thus it must be kept in mind while deciding upon the topics to be covered that the presentation of the selected topic must have scope for propagation of both gain and loss ideas to the viewers.

HIV/AIDS holds a complete spectral horizon of subtopics and components that must be conveyed to the society. The particular topics that we propose, which hold utmost importance in today's time, are:-

- Modes of transmission of HIV infection.

- Potential methods of prevention of transmission of HIV (Fig. 7).

- Crude data about current status of HIV in the society.

- Onset of symptoms in patients who contact HIV.

- Screening methods for HIV.

- Steps to be taken once a person is infected with HIV.

- Myths and stigmas about HIV/AIDS.

- Awareness and knowledge about mother-to-child

Fig. 7:

transmission of HIV/AIDS.

- Therapies available for HIV/AIDS.

- When to start and how to proceed with antiretroviral Therapies.

- Ways of prevention of further spread of HIV from an infected person.

Conclusion

Posters will continue to constitute an important medium/ instrument to combat the HIV epidemic. They are economical and an effective mode of disseminating information on all aspects of HIV to the common man. They should be aggressively utilised in campaigns against HIV.

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Treatment of Covid-19 Pneumonia with Convalescent Plasma Therapy: A Case Series

Rajnish Kaushik*, Laxmikant Tanwar**, Mit Chaudhari***, Amit Suri****

Abstract

Introduction: The entire globe is currently experiencing a pandemic by SARS-CoV-2 virus resulting in Covid-19. Management of this disease still remains largely supportive. Convalescent Plasma Therapy is still under investigation for efficacy in this disease.

Case studies: In this case series we have followed five patients who have received Convalescent Plasma Therapy along with systemic corticosteroids and two of the patients also received an antiviral agent in form of remdesivir. All five patients were above 35 years of age, all had severe Covid-19 pneumonia according to MoHFW guidelines and four patients had comorbidities (diabetes and/or hypertension).

Results and conclusion: All five patients showed clinical as well as radiological improvement in Covid-19 pneumonia after Convalescent Plasma Therapy. Inflammatory markers also started to decline following plasma transfusion. Convalescent Plasma Therapy could be an effective treatment for severe Covid-19. However, further randomised clinical trials the require to confirm its efficacy.

Key words: Covid-19, convalescent plasma therapy, case series.

Introduction

Coronavirus disease 2019 (Covid-19) caused by SARS-CoV-2 (formerly known as novel coronavirus 2019) has emerged from Wuhan city of China and infected approximately 16.3 million people, with death toll over 650,800 lives across the globe (as of 28 July 2020)[1]. The current treatment of Covid-19 is limited to general supportive care and provision of critical care[2]. The clinical data for the studies involving Covid-19 are still limited and available from China, Spain, Italy, United States of America, Germany, France and The United Kingdom. It is rapidly evolving and changing as new clinical data emerges. This will be a problem when predicting treatment outcomes.

Convalescent Plasma Therapy (CPT) is a form of adaptive immunisation. It provides an indirect method to protect a susceptible individual by granting immunity against a specific pathogen via preformed antibodies. A patient who is recovered from an infectious disease is screened for neutralising antibodies against the particular microorganism. Following identification of recovered individuals with high titers of these neutralising antibodies, convalescent plasma containing neutralising antibodies are administered to individuals with specified clinical disease. CPT has shown to reduce symptom severity as well as mortality.

Currently, Convalescent Plasma Therapy (CPT) is an attractive therapeutic option in the wake of this pandemic[3]. It has recently been suggested by Food and Drug Administration (FDA) in United States that administration and study of investigational CPT may provide a clinical benefit in treatment of Covid-19. Studies regarding effectiveness of CPT in Covid-19 are still lacking particularly in Indian settings. Here we present a case series of 5 cases of severe Covid-19 who received Convalescent Plasma Therapy. Our patients were categorised in severity grade and managed according to the Indian Council of Medical Research (ICMR) and Ministry of Health and Family Welfare (MoHFW) guidelines[5]. Clinical grading of severity of Covid-19 according to MoHFW guidelines:-

Mild: Patients with uncomplicated upper respiratory tract infection, may have mild symptoms such as fever, cough, sore throat, nasal congestion, malaise, headache. But no evidence of pneumonia.

Moderate pneumonia: Adolescent or adult with no signs of severe disease, but presence of clinical features of dyspnoea and or hypoxia, fever, cough, including SpO2 < 94% (90 - 94%) and/or respiratory rate > 24/min.

Severe pneumonia: Adolescent or adult with clinical signs of pneumonia, plus one of the following: respiratory rate > 30 breaths/min, severe respiratory distress, SpO2 < 90% on room air.

*Senior Medical Officer, ****Chief Medical Officer (NFSG) and Head of Department, Department of Respiratory Medicine, **Senior Resident, ***Post-Graduate Resident, Department of General Medicine, ABVIMS and Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 110 001.
Corresponding Author: Dr Rajnish Kaushik, Senior Medical Officer, Department of Respiratory Medicine, ABVIMS and Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 110 001. Phone: 9868534807, E-mail: drrajnishkaushik@gmail.com.

Case 1

A 60-year-old diabetic male presented with complaints of fever and breathing difficulty for 5 days. On physical examination, he was tachypnoeic with oxygen saturation of 86% on room air. Chest X-ray was suggestive of bilateral pneumonia with peripheral consolidation. SARS-CoV-2 PCR of patient came out to be positive. Patient was started on non-invasive ventilation (NIV) in view of tachypnoea and type 1 respiratory failure, empirical antibiotics, and methyl prednisolone 80 mg IV once daily. Patient also received Convalescent Plasma Therapy on the second day of admission. IV methyl prednisolone was given for 5 days. Patient became asymptomatic by 4th day of admission but he still required low flow oxygen support. By 10th day of admission, he was off oxygen, maintaining saturation at room air. Patient completely recovered with non-detectable RNA copies on PCR by 20th day of admission and discharged. His CRP levels were 210 mg/dl, 130 mg/dl and 4.5 mg/dl on day 1, day 3, and day 15 respectively.
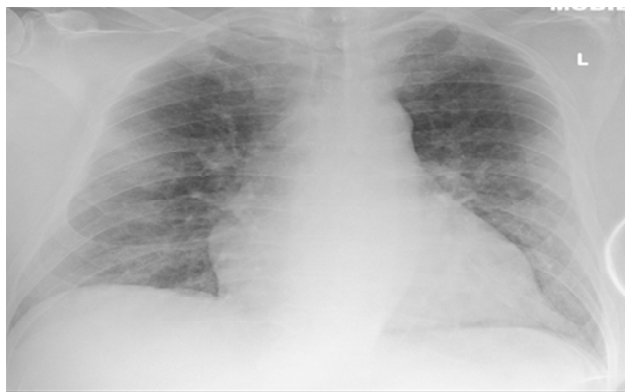
Fig. 1.1: Chest X-ray showing bilateral middle and lower zone opacities (case 1) before plasma therapy.

Fig. 1.2: Chest X-ray showing right middle and lower zone minimal peripheral opacities with clearing (Case 1) after plasma therapy.

Case 2

A 50-year-old male with diabetes mellitus, presented to the emergency department with complaints of fever for 7 days, dry cough for 4 days, and breathing difficulty for 3 days. He was tachypnoeic with saturation of 90% on room air and his chest X-ray had bilateral infiltrates with peripheral consolidation involving more than half the lung fields. PCR for SARS-CoV-2 was positive. Patient had severe category Covid-19. Patient stared on NIV with oxygen, empirical antibiotics, IV methyl prednisolone and hydroxychloroquine. Patient also received Convalescent Plasma Therapy on the day of admission. Patient was afebrile after 72 hours maintaining saturation at room air without oxygen support by the 5th day of admission. Patient was discharged after 10 days of admission period as he was asymptomatic and repeat RNA PCR was also negative. His CRP levels were 56 mg/dl, 84 mg/dl and 3.3 mg/dl on day 3, day 4 and day 5 of admission.

Fig. 2.1: Chest X-ray showing bilateral middle and lower zone consolidation (Case 2) before plasma therapy.

Fig. 2.2: Chest X-ray showing resolution (Case 2) after plasma therapy.

Case 3

A 49-year-old diabetic patient presented with complaints of fever and dry cough for 4 days and generalised weakness for 3 days. On initial assessment his saturation was 88% on

room air and chest X-ray suggestive of left middle and lower zone pneumonia with mainly peripheral consolidation. His throat and nasopharyngeal swabs turned out to be positive for SARS-CoV-2. Patient was started on oxygen inhalation and empirical antibiotics. Patient received Convalescent Plasma Therapy on second day of admission. Patient became asymptomatic and he was maintaining saturation on room air by 72 hours of admission. Patient was discharged after 15 days of admission period with non detectable viral RNA copies on RT-PCR. His CRP levels were 30 mg/dl on day 1 which decreased to 8 mg/dl and 4.8 mg/dl thereafter.

Fig. 3.1: *Chest X-ray showing bilateral opacities in peripheral distribution and left upper lobe consolidation (Case 3) before plasma therapy.*

Fig 3.2: *Chest X-ray showing no significant abnormality with resolution (Case 3) after plasma therapy.*

Case 4

A 50-year-old male with diabetes mellitus and hypertension presented with complaints of fever for 6 days and cough with breathing difficulties for 3 days. On examination, he was dyspnoeic with saturation 89% on room air and his chest X-ray was showing bilateral pneumonia with peripheral consolidation. His nasopharyngeal swab and throat swab sent for SARS-CoV-2 RT PCR, came out to be positive. The patient required

NIV support with oxygen in view of tachypnoea and type1 respiratory failure. He was started on empirical antibiotics and antiviral IV remdesivir. Patient did not respond to the standard supportive care, so on 6th day of admission he was transfused with convalescent plasma. Patient become asymptomatic with significant clinical improvement and maintaining oxygen saturation at room air by 8th day of admission. Patient was discharged after 12 days of admission period with negative RT PCR for SARS-CoV-2. His ferritin level was 1,000 ng/ml which decreased to 499 ng/ml after 5 days.
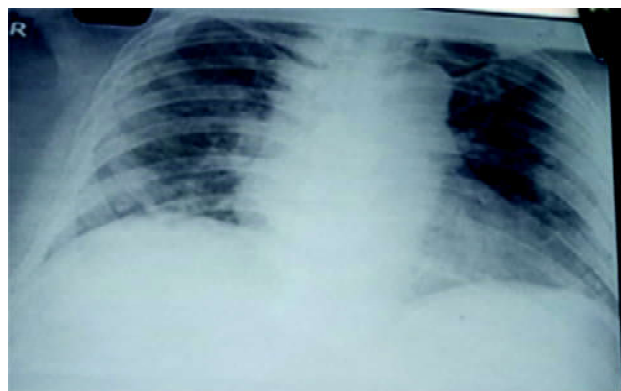
Fig. 4.1: *Chest X-ray showing bilateral peripheral consolidation (Case 4) before plasma therapy.*
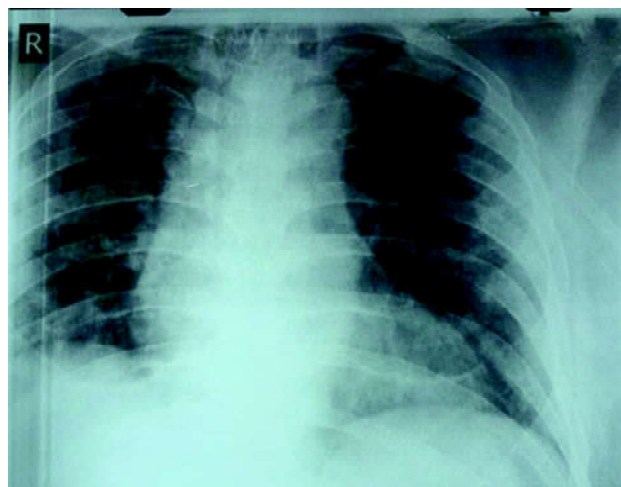
Fig. 4.2: *Chest X-ray showing resolution (Case 4) after plasma therapy.*

Case 5

A 37-year-old male came with complaints of fever since 14 days and cough with difficulty in breathing since 7 days. Patient was dyspnoeic with respiratory rate of 30 and SpO2 of 82 % on room air. Patient was given NIV support and antibiotics. Patient was administered with IV antiviral agent remdesivir. Patient was admitted on 25 July 2020. As patient did not improve on supportive treatment he was given

plasma therapy on day 3, and day 4 of admission. Gradually, he was off NIV support on day 6 and was maintaining saturation on room air on day 9 of admission. His LDH and CRP were 961 IU/L and 71 mg/dl on day 4 respectively, which

Fig. 5.1: *Chest X-ray showing bilateral patchy opacities with peripheral involvement (Case 5) before plasma therapy.*
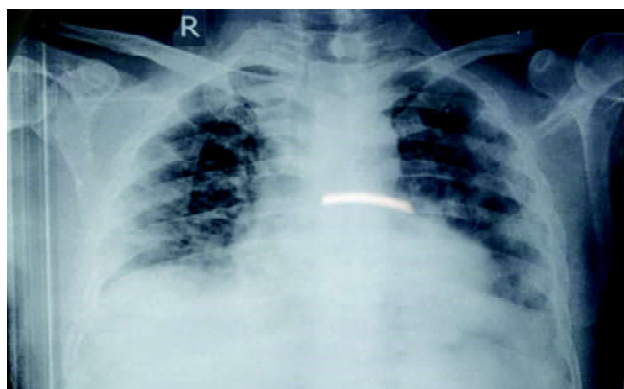
Fig. 5.2: *Chest X-ray showing bilateral peripheral opacities with clearing compared to previous X-ray (Case 5, after plasma therapy).*

reduced after Convalescent Plasma Therapy.

Discussion

In this case series, we have included five patients with severe category Covid-19 illness. All of these patients have received Convalescent Plasma Therapy between day 7 to day 20 of symptom onset. All patients turned negative on RT PCR for SARS-CoV-2 (undetectable RNA levels) following Convalescent Plasma Therapy.

A case series by Shen *et al* in March, 2020 showed improvement in clinical status and viral load after Convalescent Plasma Therapy in five patients who were critically ill Covid-19 patients. Out of these 5 patients, four patients were on mechanical ventilation[6]. However, such studies were lacking in Indian settings and we have

included patients who are either on non-invasive ventilation or oxygen support rather than invasive mechanical ventilation.

In our study, we defined severe Covid-19 as clinical signs of pneumonia plus one of the following; respiratory rate > 30 breaths/min, severe respiratory distress, SpO_2 less than 90% according to MoHFW guidelines.

Out of 5 patients, four patients had co-morbidities, four patients were 45 years or above and four patient received NIV support. All patients received empirical antibiotics, but only two received methyl prednisolone, and one patient received oral hydroxychloroquine. Out of five patients, case 4 and case 5 also received IV antiviral agent remdesivir besides the standard care. Table I is showing clinical and treatment characteristics of patients. All patients also received therapeutic anticoagulation via subcutaneous enoxaparin administration.

All patients improved clinically after Convalescent Plasma Therapy in terms of symptom recovery, temperature reduction and oxygen requirement. C-reactive protein levels and ferritin levels were obtained before and after administration of Convalescent Plasma Therapy. Quantitative levels of these inflammatory markers reduced after plasma therapy. Patients also had radiographic improvement in terms of decreased infiltrates. RNA levels by RT PCR also became undetectable after plasma therapy. Table II and Fig. 1 shows pre- and post-transfusion CRP levels. These are showing significant decline in quantitative CRP levels

In our study, two of the patients received antiviral agent, remdesivir. A Case series published in JAMA network also had 5 patients with critical Covid-19 but all of these patients
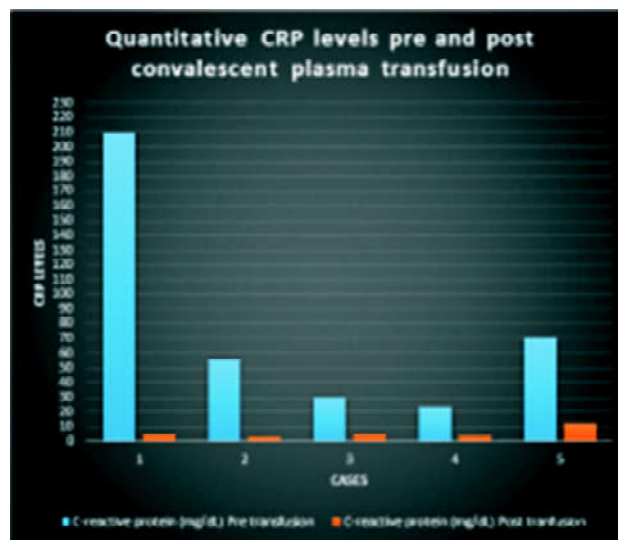
Fig. 1: *Quantitative C-reactive protein (CRP) levels before and after CPT.*

also received antiviral agents such as interferon, lopinavir-ritonavir beside convalescent plasma which may have affected outcome[6].

Table I: Clinical and therapeutic characteristics of cases.

Case	Age (years)	Co-morbidities	Duration of stay (days)	Saturation support/presentation (%)	Oxygen methyl NIV	IV predniso-lone (Y/N)	Antivirals (IV Remde-sivir)
1	60	Diabetes mellitus	20	85	NIV	Y	N
2	50	Diabetes mellitus	11	90	NIV	Y	N
3	49	Diabetes mellitus	15	88	Oxygen	Y	N
4	50	Diabetes mellitus, hypertension	11	89	NIV	Y	Y
5	37	No	12	82	NIV	Y	Y

Table II: Quantitative CRP levels pre- and post-convalescent plasma transfusion.

Cases	C-reactive protein (mg/dl)	
	Pre-transfusion	Post-tranfusion
1	210	4.5
2	56	3.3
3	30	4.8
4	23.5	4.0
5	71	12.4

Conclusion

In this uncontrolled clinical case series, five patients with severe Covid-19 who received Convalescent Plasma Therapy, showed improvement in clinical status and inflammatory markers. However, a proper clinical trial should be carried-out to further investigate effectiveness of convalescent plasma in Covid-19.

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Disseminated Brucellosis: A Diagnostic Enigma

Jency Maria Koshy*, Chris Philip Mathew**, Sunil Antony***, K Vijayakumar*

Abstract

Brucellosis is a zoonotic infection caused by gram negative bacillus of the Brucella genus. Brucellosis is transmitted through cuts and abrasions or inhalation of aerosols, or by ingestion of unpasteurised milk or milk products. Osteoarticular involvement is the most frequent complication of brucellosis. Neurobrucellosis may develop at any stage of the disease and may have widely variable manifestations. A patient with disseminated Brucellosis with subgaleal abscess involving occipital bone, neck cellulitis, meningoencephalitis and lumbar spinal discitis has been reported here.

Key words: Brucella, occipital bone abscess, cellulitis, meningoencephalitis, spinal discitis.

Introduction

Brucellosis also known as undulant fever, Mediterranean fever, or Malta fever is a zoonotic infection transmitted to humans by infected animals like sheep, cattle, goats, pigs, etc. Brucella spreads to tissues rich in elements of reticuloendothelial system, such as the joints, central nervous system, cardiovascular system and respiratory system[1]. We report a case of disseminated Brucellosis with meningoencephalitis, neck cellulitis, subgaleal abscess involving occipital bone and lumbar spinal discitis.

Case report

This 54-year-old gentleman from Kerala (India), a known patient of type 2 diabetes mellitus and systemic hypertension presented to our hospital with complaints of high grade fever with chills and backache for a duration of one week prior to admission. He was initially taken to a local hospital where the blood reports revealed thrombocytopenia (23,000/cumm). The patient developed breathlessness two days prior to admission. He had icterus, neck rigidity, diffuse oedema over the neck and bilateral infrascapular crackles.

Routine blood investigations revealed neutrophilic leukocytosis with thrombocytopenia, hyperbilirubinaemia, transaminitis, elevated C reactive protein and elevated procalcitonin (Table I). Viral markers were negative. Dengue IgM was positive. Initial arterial blood gas analysis showed metabolic acidosis with lactic acidosis. Saturation was maintained with noninvasive ventilation. A diagnosis of severe dengue was made. His platelet count gradually improved and normalised by the end of first week (Table I, II).

However, he continued to have fever and his total leucocyte count was gradually increasing (Table I, II). Blood culture sent at admission grew Klebsiella. At this point the diagnosis considered was Dengue fever with a health care-associated infection. The patient was initiated on intravenous piperacillin tazobactum, oral doxycycline, tab oseltamavir and other supportive measures. In the next few days the patient's general condition worsened and he gradually became obtunded. His fever was persisting. He was screened for other tropical fevers like scrub typhus and rickettsial infection and the reports were negative.

We proceeded with a Computed tomogram (CT) of the head which revealed brain atrophy. There were no vegetations seen on the echocardiogram.

Diffuse thickening of skin and subcutaneous tissue over the nape of neck suggestive of cellulitis was noted on ultrasonogram. CT scan of the neck revealed mild thickening of retropharyngeal soft tissue and diffuse inflammation of posterior cervical fascia and subcutaneous fatty tissue of the nape of neck. There was no obvious collection.

Antibiotics were escalated to inj. meropenem and vancomycin. Since the patient continued to be febrile and had altered sensorium, cerebrospinal fluid analysis was performed, which showed polymorphic pleocytosis (total count - 90 cells/cumm with 60% polymorphs) and the culture was sterile. By the 4th to 5th day of antibiotics, his fever came down.

Magnetic resonance imaging (MRI) of the brain and neck was done which revealed left occipital subgaleal collection and a collection in the posterior neck at C3 - C5 level (Fig. 1, 2). Even though an attempt to aspirate the collection was made, the radiologists opined that the collection was too small to be aspirated.

*Professor, **Assistant Professor, ***Associate Professor, Department of Medicine, BCMCH, Thiruvalla, Kerala - 689 103. Corresponding Author: Dr Jency Maria Koshy, Professor, Department of Medicine, BCMCH, Thiruvalla, Kerala - 689 103. Phone: 9915553245, E-mail: jencymkoshy@gmail.com.

Table I: Biochemical and haematological assays.

Parameters	Day 1	Day2	Day 3	Day 5	Day 7	Day 8	Day 9	Day 12
Haemoglobin g/dl	13.2	11.6	11.4					11.2
Total leucocyte count/mm³	12,700	13,700	13,500	14,300	17,500	20,400		12,000
Differential count	N83L10 M5E2							
Platelet/mm³		15,000	26,000	21,000	58,000	1.38 L	2.24 L	
Blood urea mg/dl			60.3					
Creatinine mg/dl	1.14	1.0	0.83	0.69		0.55		0.61
Total bilirubin mg/dl	4.66	3.98		2.86				
Direct Bilirubin mg/dl	2.91	2.5		1.51				
Total protein G/dl	5.84	5.49		5.89				
Albumin G/dl	3.13	2.87		2.71				
Aspartate aminotransferase U/L	75	75		48				
Alanine Aminotransferase U/L	54	55		43				
Alkaline phosphatase U/L	249	159		157				
Creatinine phosphokinase microgm/l	124.5		74.3					
Lactate mmol/l	5.7							
Magnesium mg/dl	2.16							
Phosphorus mg/dl	2.69							
INR	1.14							
C reactive protein mg/dl	418.4	348.4				156.3		
Procalcitonin		7.15						
Urine albumin	1+							
Glycated haemoglobin %		8.4%						
Lactate dehydrogenase U/L							813.2	

Creatinine	0.58	0.53		0.62	0.49
Total bilirubin U/L	0.76		0.61	0.59	0.6
Direct bilirubin U/L	0.26		0.24	0.2	0.17
Total protein G/dl	6.85		6.46	6.74	7.9
Albumin G/dl	2.9		2.58	2.72	3.37
Aspartate Aminotransferase U/L	90	489	34	26	16
Alanine aminotransferase U/L	153	263	86	61	13
Alkaline phosphatase U/L		188		187	179	120
Creatine phosphokinase microgm/l	81.6	38.6		101.1	50.7	40.1
T3 ng/ml (69 - 215)			131		
T4 microgm/dl (5.2 - 12.7)			7.55		
TSH micro IU/ml (0.4 - 8.9)			1.84		
INR			0.98		

Table II: Biochemical and haematological assays.

	Day 14	Day 17	Day 20	Day 23	Day 28	Day 30	Day 33	Day 50	7 month
Haemoglobin g/dl	11.9		11.9	11.4	9.7	9.4	10.6	11.7	
Total leucocyte count per mm³	9,500		12,600	14,500	11,200	9,290	7,600	10,200	
Platelets per mm³	4.21		3.64 L	3.69L	3.9 L	4.41 L		4.67 L	
Erythrocyte sedimentation rate mm/hr					40		105	90	36
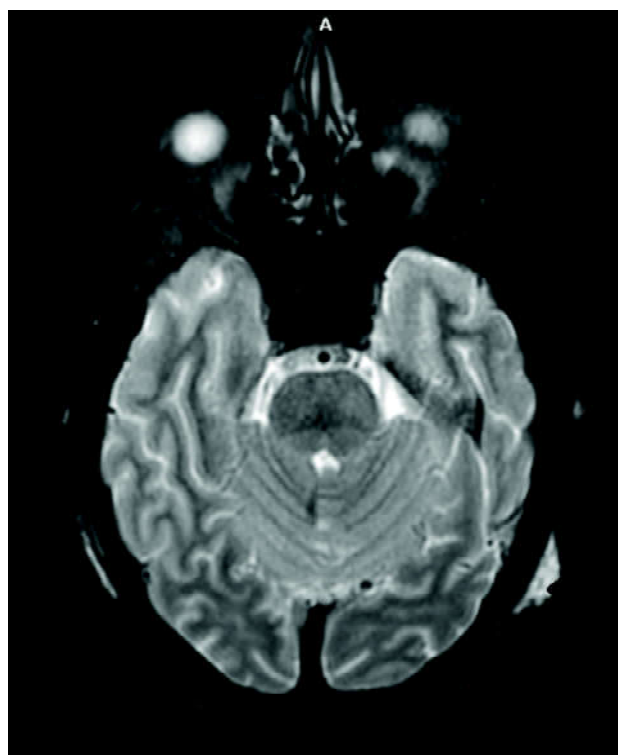
Fig. 1: MRI brain depicting subgaleal collection in left occipital bone.

The patient's general condition improved, his neck stiffness subsided and he was recuperating well.

We presumed that we were dealing with a bacterial infection since patient was responding to our treatment and we continued the intravenous injections for 14 days and then stopped. The leucocyte count improved, C-reactive protein came down, renal functions normalised and the transaminitis improved (Table II).
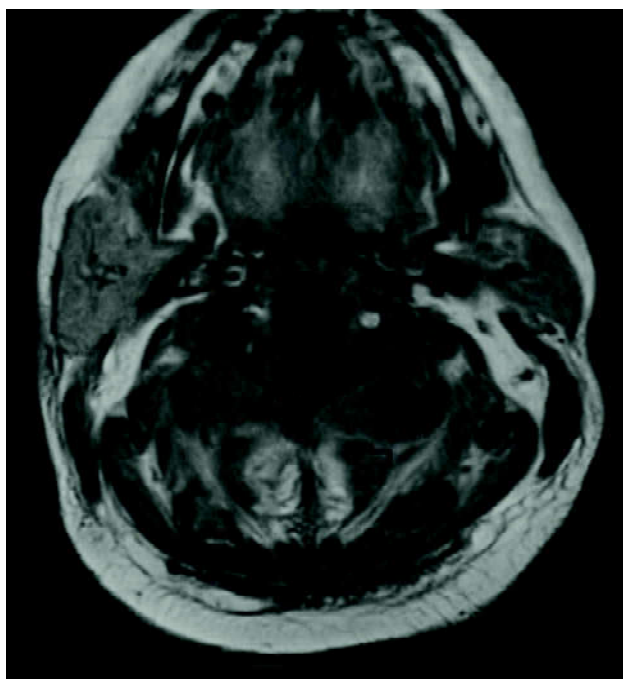
Fig. 2: MRI neck showing intermuscular collection at C3 - C5.

Within a few days of stopping the antibiotics, the patient had recurrence of fever. He was re-evaluated for the cause of fever and all the possible causes for a health care infection were addressed and remedial measures were instituted. Repeat blood cultures were sterile. Patient continued to have fever with rising leukocytosis. Even though we could not pin point any source of infection we restarted on meropenem considering a possibility of health-care associated Gram-negative bacterial infection and he became afebrile.

Patient was mobile by then and he noticed that he had severe back ache with pain radiating to the right lower limb. Clinical examination revealed tenderness at L5. MRI of lumbosacral spine revealed L5-S1 infective spondylodiscitis with epidural soft tissue (Fig. 3, 4). A CT guided biopsy of the spinal cord lesion was done. AFB (acid fast bacilli) smear and TB PCR was negative. Brucella IgM antibody was borderline positive. Brucella agglutination also was reactive. Focal areas of small collection of epitheliod cells depicting an attempted granuloma were noted on histopathology (Fig. 5). Pathologists opined that these granulomas can be consistent with Brucellosis in the given clinical setting. On reviewing the history, we learnt that he consumed boiled milk and had no history of exposure to cattle. Even though Kerala state of India is not an endemic area, it was noted that he was earlier working in a Middle-Eastern country which is known to be an endemic area for Brucellosis.

Brucella's susceptibility to Meropenem could possibly
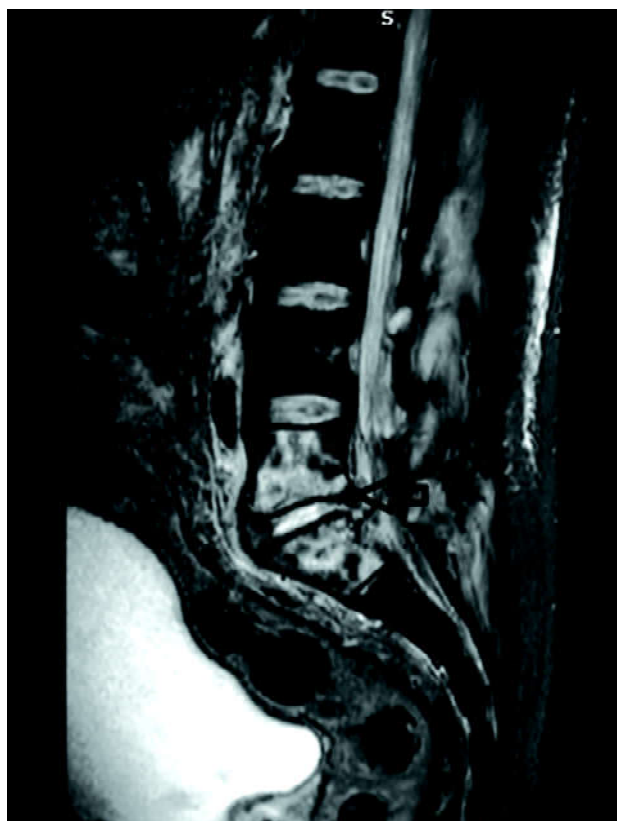
Fig. 3: MRI of the lumosacral spine revealing L5 S1 discitis with paraspinal collection.
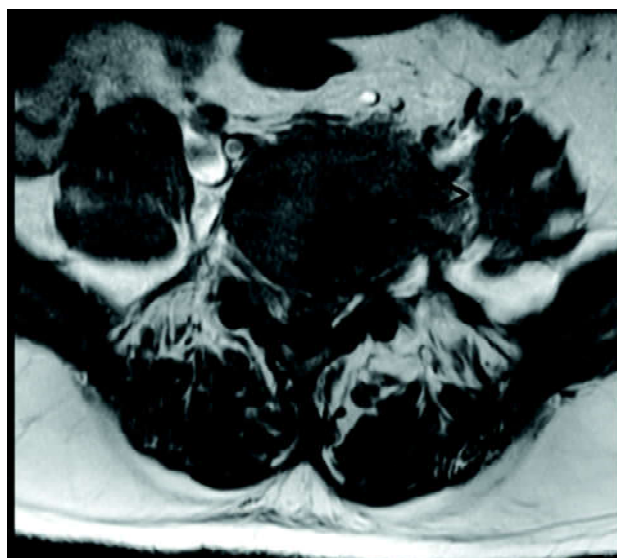
Fig. 4: Paraspinal collection.

explain the defervescence of fever while on Meropenem. We stopped Meropenem and initiated him on intramuscular streptomycin (21 days), tab doxycycline, and tab rifampicin. His condition gradually improved. He remained afebrile thereafter. An MRI done 6 weeks later revealed reduction
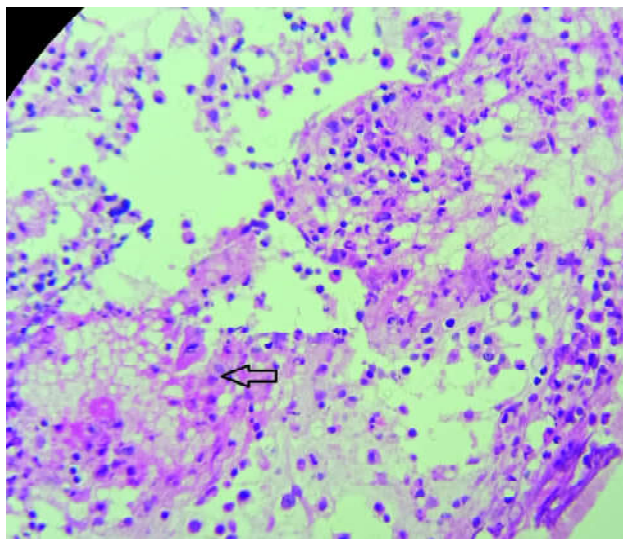
Fig. 5: *Histopathology showing focal areas of collection of epitheloid cells.*

in oedema and reduction in epidural soft tissue at L5 - S1. Doxycycline and rifampicin were given for a total duration of 12 weeks. There was resolution of neurological deficit and radicular pain and his ESR returned to normal. He was further followed-up for 6 months and he continued to remain asymptomatic.

Looking back, we could assemble the pieces of the jigsaw puzzle. This patient indeed had presented with dengue infection as he had presented with significant thrombocytopenia in the backdrop of dengue serology positivity. His platelets improved within a week which was what we expected in dengue infection. We did not refute the presence of Klebsiella bacteraemia at presentation as he came in from another health care facility. However, that did not explain the neck cellulitis, meningoencephalitis, and subgaleal collection. Brucellosis is a multisystem disorder which can cause osseous involvement including skull bone, skin and soft tissue infection, meningoencephalitis, and spinal discitis. Clinical resolution following therapy for brucellosis reiterated our diagnosis.

Discussion

This patient had a multisystem involvement of brucellosis including osseous, cutaneous, and nervous system involvement.

The various neurological involvement of Brucella noted in literature are encephalitis, meningoencephalitis, radiculitis, myelitis, peripheral and cranial neuropathies, subarachnoid haemorrhage, and psychiatric manifestations[2, 3]. Signs and symptoms of meningeal involvement are nonspecific in neurobrucellosis and meningeal signs are infrequently

present[4]. Our patient had meningoencephalitis. According to Kochlar *et al* the criteria necessary for definite diagnosis of neurobrucellosis are: (i) neurological dysfunction not explained by other neurologic diseases, (ii) abnormal CSF indicating lymphocytic pleocytosis and increased protein, (iii) positive CSF culture for *Brucella* organisms or positive *Brucella* IgG agglutination titre in the blood and CSF, and (iv) response to specific chemotherapy with a significant drop in the CSF lymphocyte count and protein concentration[5].

Osteoarticular involvement is the most frequent complication of brucellosis, and can occur in 10% to 85% of the patients infected with the disease[6]. The spine is one of the most common organs involved in brucellosis infection with a rate of 2% - 54%, and the lumbar vertebrae are the most frequently affected[7]. Sohn *et al* reported an unusual case of occult Brucella osteomyelitis involving the skull[8]. This patient had subgaleal abscess involving occipital bone and lumbar discitis with radiculopathy.

Chronic ulcerations and subcutaneous abscesses have also been described in brucellosis[9]. The neck abscess in this patient resolved completely with treatment.

The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time. Uncomplicated cases can be treated with doxycycline 100 mg twice a day for six weeks + streptomycin 1 g daily for two to three weeks or doxycycline 100 mg twice a day for six weeks + rifampicin 600 - 900 mg daily for six weeks[10]. IDSA (Infectious disease society of America) also suggest doxycycline with rifampicin for treatment of Brucella osteomyelitis[11]. The WHO recommends that drugs which penetrate blood brain barrier like rifampicin or co-trimoxazole should be added to the standard regimen of doxycycline plus streptomycin in the treatment of central nervous system complications of brucellosis. The optimal duration of treatment for neurobrucellosis has not been determined; however, most authorities recommend a minimum of six to eight weeks, and possibly longer, depending on the clinical response[10].

Conclusion

Brucella is a multisystem disease with variable presentation. A high index of suspicion is required to diagnose Brucellosis. Brucellosis should be considered in the differential diagnosis of a multisystem involvement in patients returning from an endemic area.

BCMCH for the help provided in diagnosing this case.

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Recurrent Seizure: An Unusual Presentation of Bartter Syndrome in an Adult Female

Saptarshi Mukhopadhyay*, Sarmishtha Mukhopadhyay**, Sarbani Sengupta***, Bhaskar Ghosh****

Abstract

A young lady presented with recurrent seizure. Examination revealed features suggestive of tetany. Investigation showed hypokalaemia, severe hypocalcaemia and hypercalcuria, hyper-reninaemia, hyperaldosteronism, and metabolic acidosis. Adult onset, severe hypocalcaemia along with other biochemical features points towards type V Bartter syndrome. Seizure is an unusual presentation of Bartter syndrome making this case reportable.

Key words: Bartter syndrome, hypokalaemia, hypocalcaemia, metabolic alkalosis, seizure.

Introduction

Bartter syndrome was originally described by Bartter and colleagues in 1962[1]. It is an autosomal recessive renal tubular disorder characterised by hypokalaemia, hypochloraemia, metabolic alkalosis, hypercalcuria and hyporeninaemia with normal blood pressure[2]. Bartter's syndrome mostly presents the in neonatal period with hypokalaemic metabolic alkalosis. Initial presentation of Bartter syndrome in adults is relatively less common. Here we report a case of Bartter syndrome in an adult female who presented with intractable seizure.

Case report

A 27-year-old female presented in emergency room in a drowsy state with history of generalised tonic clonic seizure one hour prior to admission. She had history of similar episodes twice in the last 1 month and was prescribed levetiracetam 500 mg thrice daily after the first episode. There was no history of headache, vomiting, visual disturbance, or fever in the recent past. Relatives stated that she was having perioral numbness and leg cramps for the last 2 months. She was not on any regular medication except the antiepileptic and did not have any history of addiction or substance abuse. She was a non-vegetarian and family history was non-contributory.

On clinical examination, the patient was found to be drowsy, but arousable. Her vitals were stable. Pupils were of normal size and reacting. There was no cranial nerve palsy, motor or sensory deficit. Meningeal signs were absent and reflexes were normal. Next morning, the patient recovered from postictal drowsiness. During measurement of blood

pressure, the patient developed spasmodic contraction of the hand. So, the patient was examined for Trousseau's sign which was found to be positive. Chvostek's sign was also positive. General and systemic examination including neurological findings were otherwise non-contributory.

Laboratory investigations revealed: Total leucocyte count: 4,600/mm³, differential leucocyte count:- neutrophils: 74%, lymphocytes: 22%, eosinophils: 2%, monocytes: 2%, basophils: 0%, fasting plasma glucose: 102 mg/dl, blood urea: 24 mg/dl, serum creatinine: 0.9 mg/dl, total bilirubin: 1.0 mg/dl, aspartate transaminase: 39 IU/L, alanine transaminase: 42 IU/L, alkaline phosphatase: 120 IU/L, total protein: 7.3 g/dl, serum albumin: 4.0 g/dl, serum globulin: 3.3 g/dl, serum sodium: 136 meq/l (normal value: 135 - 145 meq/l), serum potassium: 2.0 meq/; (normal value: 3.5 - 5.0 meq/l), serum calcium: 6.6 mg/dl (normal value: 8.5 - 10.2 mg/dl), serum magnesium: 2.1 mg/dl (normal value: 1.7 - 2.3 mg/dl), serum phosphate: 3.3 mg/dl (normal value: 2.5 - 4.5 mg/dl), TSH: 2.34 mIU/L, 25 hydroxy vitamin D: 32 ng/ml (normal value: > 30 ng/ml), serum iPTH: 48 ng/l (normal value: 10 - 65 ng/l), 24 hours urinary potassium: 35 mmol/day (normal value: < 15 mmol/day, 24 hours urinary calcium: 923.5 mg/24 hours (normal value: 100 - 300 mg/24 hours), plasma renin: 35.2 ng/ml/hour (normal value: 06 - 4.3 ng/ml/hour), plasma aldosterone: 295 pmol/l (normal value: 55 - 250 pmol/l). Arterial blood gas analysis:- pH: 7.63, Pa O_2: 79 mmHg, Pa CO_2: 41 mmHg, HCO_3: 39 mmol/l.

MRI brain was normal, and EEG was suggestive of seizure disorder.

On the basis of hypokalaemia, metabolic alkalosis, hypocalcaemia, hypercalciuria, normal blood pressure,

*Senior Resident, **Physician and Endocrinologist, ***Consultant Endocrinologist, Department of General Medicine, BR Singh Hospital and Centre for Education and Research, Kolkata - 700 002, Eastern Railway; ****Consultant Neurologist, Department of General Medicine, MM Medical Centre, Kolkata - 700 002.
Corresponding Author: Dr Sarmishtha Mukhopadhyay, Senior Resident, Department of General Medicine, BR Singh Hospital and Centre for Education and Research, Kolkata - 700 002. Phone: 9002021567, E-mail: drsarmishtha67@gmail.com.

hyperreninaemia and hyperaldosteronism, the patient was diagnosed as a case of adult variant of Bartter syndrome. She was treated with calcium gluconate infusion and oral potassium chloride syrup along with antiepileptic drug. After 72 hours, the patient stabilised and there was no further seizure. On 4th day, serum potassium was 3.5 meq/l and serum calcium was 8.0 mg/dl. Spironolactone 300 mg/day, indomethacin 75 mg/day and oral calcium 500 mg thrice daily were added before discharge on 5th day.

Discussion

Bartter syndrome is an autosomal recessive renal tubular disorder characterised by normal blood pressure, hypokalaemia, metabolic alkalosis, hyperreninaemia, secondary hyperaldosteronism, and usually high level of prostaglandin E2 (PGE2). Prevalence of Bartter syndrome is 1 in 1,000,000[3]. The low prevalence may be in part due to lack of diagnosis because of prenatal or neonatal death.

The primary defect is an impairment of one of the transporters involved in sodium chloride absorption in the loop of Henle and distal convoluted tubule[1,4]. It produces a clinical disorder similar to that with chronic loop diuretic ingestion.

The underlying pathology results in excessive urinary losses of sodium and chloride leading to volume depletion followed by activation of renin-angiotensin-aldosterone system[5].

Excessive distal sodium delivery increases distal tubular sodium reabsorption and exchange with electrically equivalent potassium or hydrogen ion which in turn, promotes hypokalaemia. At the same time, this promotes inadequate exchange of bicarbonate. Combined hypokalaemia and excessive bicarbonate retention lead to metabolic alkalosis.

Persons with Bartter syndrome also have hypercalciuria[6]. Normally, reabsorption of negative chloride ions promotes a positive voltage in the lumen, driving paracellular positive calcium and magnesium absorption. Dysfunction of chloride transporters in the loop of Henle prevents urine calcium reabsorption leading to hypercalciuria. Excessive urine calcium excretion may be one factor responsible for nephrocalcinosis observed in a few patients.

Along with volume depletion, renal release of prostaglandins (PGE2) result in relatively normal blood pressure in spite of secondary hyperaldosteronism[7].

Bartter syndrome is classified into 5 types with the following genetic defects[8,9,10]:-

1. Type I: Defective function of Na-K-2Cl (NKCC2, encoded by *SLC12A1*) co-transporter.

2. Type II: Defective function of luminal potassium channel (ROMK encoded by *KCNJ1*).

3. Type III: Defective function of basolateral chloride channel (ClC-Kb, encoded by *CLCNKB*).

4. Type IV and IVb: Defective function of ClC-Ka and ClC-Kb (single mutation affecting Barttin subunit in IV and double mutation affecting both channels in IVb).

5. Type V: Gain of function mutation of calcium sensing receptor (CaSR).

Type I, II, IV and IVb Bartter syndrome present in antenatal period and the patients usually die in neonatal period. Type III and type V Bartter syndrome are less severe and present in adults with hypokalaemia, metabolic alkalosis, and hypercalciuria.

Type V Bartter syndrome is also called autosomal dominant hypocalcaemia (ADH). It is caused by gain of function mutation of calcium sensing receptor (CaSR) gene (ADH type1) or gain of functions mutations of G alpha 11, a key mediator of CaSR signalling (ADH type2)[11-13]. Activation of CaSR blunts potassium efflux through ROMK channel and also may reduce activity of Na-K-2Cl co-transpoter. Sporadic *de novo* mutation of CaSR have also been reported. Most of the patients are asymptomatic although few patients present with seizure[14]. The biochemical features include: serum calcium: 6 - 8 mg/dl, sometimes as low as 5 mg/dl, inappropriately normal serum PTH and high or high normal urinary calcium excretion. Severe hypocalcaemia with resultant seizure along with other relevant biochemical picture in an adult patient probably leads to the diagnosis of type V Bartter syndrome in this patient although genetic study could not be done.

Non-steroidal anti-inflammatory drugs (NSAIDS) which inhibit prostaglandin production and potassium sparing diuretics which block sodium-potassium exchange in distal tubule are the main options of therapy. Potassium and calcium supplementation are the other modalities of treatment. Our patient responded to this combined therapeutic approach along with antiepileptic drug.

Conclusion

Adult onset Bartter syndrome itself is a rare entity. Its presentation with seizure due to hypocalcaemia is even rarer which makes the case reportable. The take home message is to look for hypokalaemia and metabolic alkalosis in a patient with symptomatic or asymptomatic hypocalcaemia, otherwise we may miss the diagnosis of a rare disease like Bartter syndrome.

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RS3PE in a Young Female

Harpreet Singh*, Somdatta Giri**, T Seetam Kumar***, Anubha Garg****, Amit Gaider**

Abstract

A 20-year-female presented with acute onset pain and swelling of hands and feet. This was atraumatic and associated with early morning stiffness but not accompanied by rash, Reynaud's phenomenon, fever. Her systemic examination was unremarkable. She was sereonagative for rheumatoid factor, ANA, and her thyroid profile was also normal. Doppler ultrasonography revealed oedema around extensor tendons of hands. She responded dramatically to low-dose steroid. A diagnosis of Remitting Seronegative Symmetrical Synovitis with Pitting Oedema (RS3PE) is made which is very uncommon to find out in young age.

Key words: Remitting seronegative symmetrical synovitis with pitting oedema (RS3PE), rheumatoid arthtritis, polyarthritis.

Introduction

Remitting Seronegative Symmetrical Synovitis with Pitting Oedema (RS3PE), a concept that was first advocated by Mc Carty *et al*[1] in 1938, is a rare syndrome which is a subset of the seronegative symmetric polyarthtitis of older people – predominantly seen in males. It was formerly considered as a subset of rheumatoid arthritis (RA), but it is now regarded as a distinct disease. We present a case with typical features of RS3PE in a 20-year-old girl.

Case report

A 20-year-female student presented with atraumatic pain and swelling of both hands (involving wrist, metacarpophalangeal and proximal interphalangeal joints) and feet for about three months. This was accompanied with early morning stiffness lasting more than two hours. There were no accompanying features like rash, Raynaud's phenomenon, fever, burning micturition, urethral discharge, or diarrhoea. For the pain and swelling of hands and feet she was prescribed NSAIDs, but with no relief.

Clinical examination revealed marked pitting oedema of the dorsal surface of hands and feet extending up to wrist and ankle (Fig. 1). Swelling was tender but not warm to touch. There was limitation of movements of wrist and MCP joints, and the joints of feet. Her systemic examination was unremarkable.

Laboratory investigations revealed Hb: 11.9 g/dl; TLC: 14,400/mm^3; platelets: 360,000 h/mm^3; ESR: 36 mm in the 1st hour; CRP: > 90 IU/dl. Her LFT, RFT, urine microscopy were normal. Serology was negative for rheumatoid factor, anti CCP, ANA, hepatitis B and C and HIV. Thyroid profile was normal. Ultrasound abdomen and chest X-ray were normal. X-ray of both hands was normal, without any evidence of erosion (Fig. 2). Colour Doppler sonography of small joints of hands revealed extensor tenosynovitis (Fig. 3). CECT chest was normal (done to rule-out a secondary cause such as sarcoidosis or occult malignancy).

A diagnosis of RS3PE was made. The patient was started on oral prednisolone 20 mg/d. She responded extremely well with resolution of swelling and pain within a week and
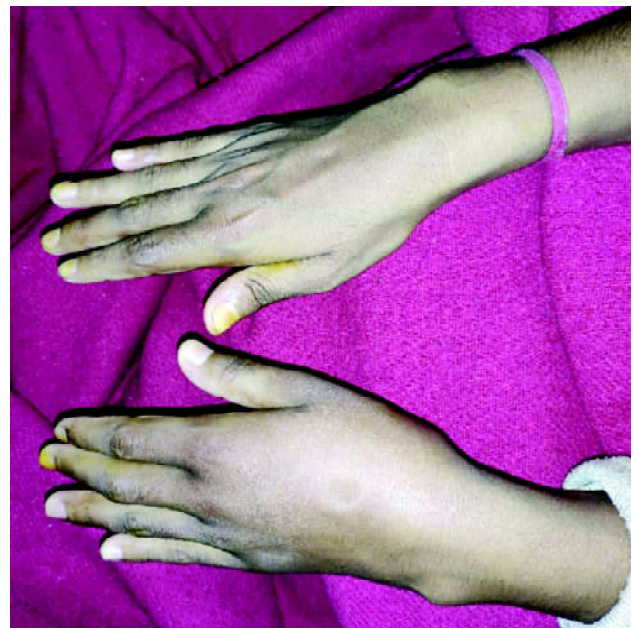
Fig. 1a: *Showing swelling of hands.*

*Senior Professor and Head, **Junior Resident, ***Associate Professor, Department of Medicine, ****Junior Resident, Department of Radiology, Pandit BDS PGIMS Rohtak, Haryana.*
Corresponding Author: Dr Somdatta Giri, Junior Resident, Department of Medicine, Room No. 262 PG Hostel, Pandit BDS PGIMS, Rohtak - 124 001, Haryana. Phone: 9002004026, E-mail: somdattagiri@gmail.com.

Fig. 1b: Showing swelling of feet.

Fig. 2: Skiagram of both hands AP view.
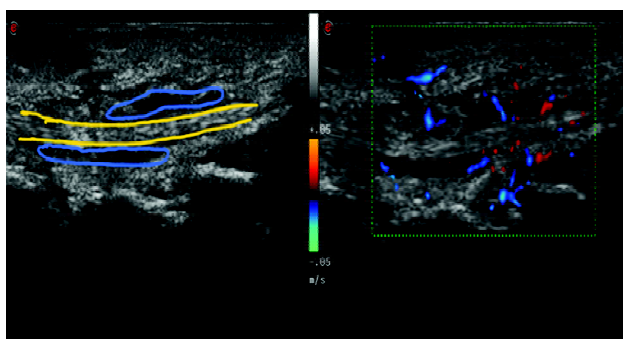
Figure 3a: Sonographic image on longitudinal view (on the left) depicting fluid (encircled by blue-line) along the extensor digiti minimi tendon (demarcated as two yellow-lines) and also vascularity on colour map (on the right) suggesting active tenosynovitis.
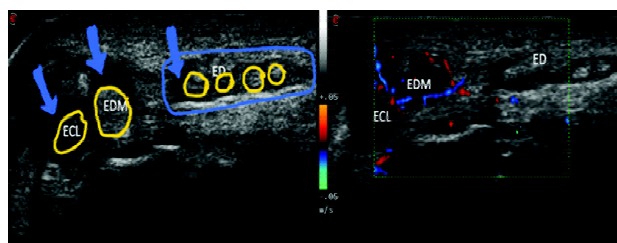
Figure 3b: Sonographic image on axial section (on the left) showing fluid (blue arrows) and vascularity (on the right) along extensor group of tendons (yellow circles).

EDM- extensor digiti minimi, ED- extensor digitorum, ECL- extensor carpi ulnaris.

steroid was gradually tapered-off over three months. Till the time of reporting, our patient did not show any sign of relapse.

Discussion

The diagnosis of RS3PE is not easy as it is always hindered by lack of definitive diagnostic criteria and presence of other much common rheumatological diseases that they may mimic. Following a retrospective multicentred study, Olive et al[2] proposed the following diagnostic criteria for this syndrome:-

- Bilateral pitting oedema of hands
- Sudden onset of polyarthritis
- Age > 50 years
- Seronegative for Rheumatoid Factor.

Since Mc Carty's original description, over 150 case of RS3PE have been described[3]. Most of them fulfilled the original criteria. However, many cases do not fit the criteria adding new dimensions to this entity. RS3PE has been infrequently reported in young age (< 30 years)[4,5,6].

The association of pitting oedema and arthritis of the hands is very rare. It is very suggestive of RS3PE syndrome. Onset may be rapid, in less than a month. The clinical picture is characterised by a florid pitting oedema of the dorsum of the hands, which may also be present in the feet. Wrist joint movement is often limited and there may be a small effusion in the knee joints. Pain involves the wrists, the MCPs and IPs as well as the flexor tendon sheaths of the fingers. Shoulder girdle pain is also frequently reported, but the pelvic girdle is seldom involved.

Laboratory tests typically demonstrate an inflammatory state with increased erythrocyte sedimentation rate and C-reactive protein, discrete inflammatory anaemia and hypoalbuminaemia. There is seronegativity for rheumatoid factor and ANA, positive for HLA B7. In cases where synovial fluid analyses were carried-out, leucocyte counts were usually lower than in rheumatoid arthritis.

Radiography of the hands and wrists show soft tissue oedema and generalised osteopenia. Bone erosions are absent. MRI studies suggest distinct extensor tenosynovitis as the principle lesion[7].

The immune pathogenesis of RS3PE is still in the dark. The clustering of patients from rural areas and the seasonal variation points towards its infectious or para-infectious origin. Tuberculosis, Parvovirus B19, *Streptobacillus moniliformis*, *E. Coli*, *C. jejuni*, Mycoplasma have all been listed[8]. But causal relationship has not been established. One study has suggested that VEGF as a major contributor to polysynovitis by increasing hypervascularity and vessel permeability, which by turn lead to subcutaneous oedema[9]. IL-6 has been found to be elevated in synovial fluid. Vasculitis of lymphatics has also been postulated[10], but, lymphoscintigraphy studies showed no reduction of axillary lymphnode radioactivity, indicating normal lymphatic function.

There has also been association with HLA B7 and A2 haplotype, however their role in inheritance is still uncertain due to paucity of documented cases. There have been reports of apparently classic RS3PE syndromes that were complicated by connective tissue diseases such as polyarteritis nodosa or other vasculitides. Pitting oedema typical of RS3PE was reported in lupus, ankylosing spondylitis, and temporal arteritis. Patients with seropositive rheumatoid arthtritis can have similar manifestation but bony erosion is invariably present and they relapse after steroid withdrawal.

RS3PE syndrome has also recently emerged as a potential paraneoplastic syndrome. Cases of gastric carcinoma, endometrial carcinoma, and pancreatic carcinoma have been reported. In all cases, complete remission was observed after resection of the tumour, indicating a true paraneoplastic syndrome.

The association of arthritis and oedema of the hands is fairly uncommon and should lead us to consider certain diagnoses when confronted by such cases. RS3PE is not the sole diagnosis.

● Mixed connective tissue disease can present with arthralgia and oedema of hands (including sausaging of digits) with Raynaud's phenomenon (absent in RS3PE).

● Both reactive arthritis and psoriatic arthropathy may present with firm, non-pitting, oedematous hand involvement which is asymmetrical (unike symmetrical involvement in RS3PE).

● Oedema of lower limb with sparing of upper limb has been reported with seronegative spondyloarthropathy.

● Oedema of the hands can be the presentation of reflex sympathetic dystrophy that may be bilateral. Exquisite pain aggravated by active and passive mobilisation, vasomotor and skin alterations, absence of true arthritis, presence of predisposing factors such as myocardial infarction, stroke, or use of barbiturates, and the absence of systemic inflammation, will generally lead to the correct diagnosis.

● Rarely in rheumatoid arthritis, oedema of the hands has been observed during severe flare-ups and hence attributed to changed capillary permeability secondary to the diffuse inflammatory process. Unilateral oedema resulting from a capsular rupture at the wrist, similar to the Baker cyst rupture in the knees, has also been reported. Unlike the prompt response observed in RS3PE, these cases of rheumatoid lymphoedema do not respond well to second-line treatment or corticosteroids. Demonstration of erosions and high leucocyte counts in synovial fluid favours the diagnosis of rheumatoid arthritis.

● Polymyalgia rheumatica is a disease of elderly (like RS3PE) but less likely to present with oedematous extremities – especially upper limbs (unlike RS3PE) – and is prone to recur.

Prognosis is excellent. Duration of treatment is usually less than a year (average 6 - 18 months). Response to NSAID is not good. Low-dose prednisone (10 to 15 mg/day) was found to be effective with a rapid and spectacular effect. RS3PE patients who have underlying malignancy respond poorly to steroid. Surgical removal of tumour – if possible – shows complete remission. Those who poorly respond, hydroxylchloroquine can be added as a disease modifying agent. The literature is silent about the response with other DMARDs in RS3PE. Tocilizumab, a monoclonal antibody against IL-6 receptor, is effective in selected patients with high IL-6 level and can be used in steroid-dependant/refractory cases. Low-grade flexion contractures may develop on wrists and fingers that may sometimes be permanent.

In the present case, our patient responded to steroid.

Conclusion

RS3PE is an uncommon syndrome but should be considered in a typical clinical scenario as it has an excellent prognosis. The mechanism since understood cannot explain why the elderly are prone to this syndrome. And one must be suspicious of this syndrome in the younger age group too. This case adds clinical aspects to reconsider the 'age' criteria mentioned in the diagnostic criteria as it does not add value to the pathophysiology of the disease, or have any prognostic implications.

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Digital Gangrene: A Rare Complication in Scrub Typhus

Mahesh Dave*, Saurabh Jain**, Heer Nath**, Nagraj**

Abstract

Scrub typhus or bush typhus is one of the most common zoonotic diseases which is predominantly endemic in Asia pacific region. This disease is predominantly seen in the monsoon and post-monsoon season and has high fatality rate. Clinical presentation of scrub typhus may be variable which range from mild febrile illness to multi organ dysfunction syndrome and possible pathophysiology may be due to focal to diffuse vasculitis. Focal vasculitis of digital vessels may lead to digital gangrene which may be a rare association in scrub typhus patients. Hence, we are reporting a patient of scrub typhus who developed digital gangrene during the acute phase of the disease.

Key words: Scrub typhus, gangrene, vasculitis.

Introduction

Scrub typhus is also known as Tsutsugamushi disease or Bush typhus, is a disease caused by a intracellular gram negative organism *Orrientia* (formerly Rickettsia) *tsutsugamushi*. It is one of the most common zoonotic diseases which is transmitted to humans by an arthropod vector of the Trombiculidae family. The name tsutsugamushi is derived from two Japanese words – "Tsutsuga" means small and dangerous, and "mushi" means insect or mite (chiggar)[1]. It affects people of all the ages and is a serious public health problem in the Asian pacific region.

The disease is predominantly seen in monsoon and post-monsoon season (July to November) and has a high fatality rate. Around a billion people are at risk and nearly a million cases have been reported every year[2]. Mite can serve as both the vector and the reservoir.

Scrub typhus is seen worldwide but it is endemic to a part of world known as "tsutsugamushi triangle," which extends from northern Japan and eastern Russia in the north to northern Australia in the south and to Pakistan and Afghanistan in the west[3]. Scrub typhus is often acquired during occupational or agricultural exposures[4].

Scrub typhus may present clinically as mild disease – such as fever, skin rashes, thrombocytopenia, and lymphadenopathy – to more severe forms such as meningitis, meningoencephalitis, myocarditis, acute renal failure, multi-organ dysfunction, acute hepatic failure, and focal or pan digital gangrene.

Focal or pan digital gangrene is one of the rare complications which may be observed during the acute phase or in the recovery phase of scrub typhus patient and caused due to focal or disseminated vasculitis or perivasculitis of digital blood vessels. Hence, we are reporting a patient of scrub typhus who developed digital gangrene during the acute phase of the disease.

Fig. 1a: *Dry gangrene involving digits of right hard.*

Case report

A 60-year-old female (housewife) admitted to our medical ICU with history of high-grade fever and chills, headache,

*Senior Professor and Unit Head, **Junior Resident, Department of Medicine, RNT Medical College, Udaipur - 313 001, Rajasthan. Corresponding Author: Dr Mahesh Dave, Senior Professor and Unit Head, Department of Medicine, RNT Medical College, Udaipur - 313 001, Rajasthan. Phone: 9460718058, E-mail: mdaveleena@gmail.com.

Fig. 1b: *Dry gangrene involving digits of Left Foot.*

Fig. 1C: *Dry gangrene involving tip of nose.*

generalised bodyache, nausea and vomiting for the last fifteen days and shortness of breath for the last 2 days for which she consulted a physician who prescribed antibiotics, antipyretics with anti-histaminics for five days

but without any improvement, and hence was referred to our centre. Her examination revealed anaemia, jaundice with fever 101° F orally. Rest of the physical examination was within normal limits and there was no eschar mark. Systemic examination revealed tachypnoea (20 per minute) with occasional crackles present in the right lower part of the chest. There was no organomegaly, no signs of meningeal irritation, and all peripheral pulses were palpable and normal. Clinically, we made our differential diagnosis as acute febrile illness without organomegaly suspected to be malaria, scrub typhus, or dengue fever and this patient was investigated extensively: Haemogram (Hb: 9.6 g/dl, WBC: 20,600/cumm, platelet count: 59,000/cumm). Her serum urea was 145 mg/dl, creatinine: 2.19 mg/dl, bilirubin (total: 1.8 and direct: 1.3 mg/dl), SGOT: 102 U/L, SGPT: 65 U/L, ALP: 271 U/L, urine examination was normal, and HBsAg, HCV, HIV status was found to be negative. She was further investigated to establish an aetiological diagnosis for this febrile illness with multi-organ dysfunction syndrome in the form of malaria parasite quantitative buffy coat test (MP QBC) and malaria parasite slide test which was negative, dengue NS1, IgG and IgM Dengue was also negative but IgM by Elisa method for Scrub typhus was positive. Rest of investigations included X-ray chest, arterial blood gas analysis, ultrasonography abdomen, electrocardiography, echocardiography and were within normal limits. Patient was put on doxycycline 100 mg twice a day and azithromycin 500 mg once a day therapy with symptomatic management. During the Intensive care unit stay on second day, this patient started complaining of severe burning pain in both upper limb fingers for which she was put on analgesics with partial improvement but on next day she started complaining of blackening of fingers in the upper and lower limbs as well as tip of the nose; and when we examined, we discovered dry gangrene in upper and lower limb fingers and tip of the nose which progressed over the next 2 days with ulcer formation. She was examined further and all her peripheral pulses including cardiovascular system examination was found to be normal. Suspecting vasculitis as a cause for the gangrene, vasculitis work-up was done which included ESR, C-reactive protein, anti-nuclear antibody (ANA), Rheumatoid Factor (RF), anti-neutrophilic cytoplasmic antibody (C and P-ANCA), were negative. Vascular surgeon's opinion was taken and he advised colour Doppler study of upper and lower limb arteries which was found to be normal. Patient was treated with appropriate antibiotics with low molecular weight heparin and followed-up over a period of two weeks. She had partial improvement – became afebrile, and the gangreneous part was partially reverted to normal. Her liver and kidney function tests reverted to normal and hence she was discharged and advised her to follow-up

with medical and cardio-thoracic surgery out-patient department.

Discussion

Scrub typhus is one of the common zoonotic diseases which has re-emerged in the last few years in various part of India and also in southern Rajasthan. Its incidences are showing an incremental trend in last few years. It shows seasonal variation and predominant cases have been observed in the monsoon and post-monsoon season between July and November. Scrub typhus may present as a mild febrile illness to much severe fatal complications like ARDS, myocarditis, meningoencephalitis, acute kidney injury, hepatic dysfunction, multi-organ dysfunction, and focal-to-extensive vasculitis.

Digital gangrene (partial or complete in all digits) is a sign of systemic disease which can be seen in infective disease such as syphilis, leprosy, viral hepatitis, HIV, meningococcemia, leptospirosis, and other non-infectious diseases like autoimmune disease such as polyarteritis nodosa, Wegener's granulomatosis, Churg–Strauss syndrome (which usually involves medium sized arteries), and systemic lupus erythematosus, RA, scleroderma, and antiphospholipid syndrome, which may commonly involve small vessels and can present with digital gangrene[5].

Gangrene is an uncommon complication observed in rickettsial disease and very few cases have been reported worldwide yet[6]. The distribution of gangrene may be due to selective proliferation of these bacteria in the cooler body regions leading to vasculitis, perivasculitis, tissue hypoperfusion, and gangrene development[7]. Hence, before labelling as a case of rickettsial disease-induced gangrene, we should rule-out all possible causes such as infectious as well as autoimmune conditions.

Conclusion

Scrub typhus is a re-emergent zoonotic disease in the Indian subcontinent. It is one of the most common causes of acute febrile illness in the rainy and post-rainy season, and clinically presents with mild form like acute febrile illness to a more severe dreadful complication like vasculitis. Vasculitis may be diffuse – involving multiple organs like liver, kidney, CNS and hematopoietic system – and may present as multi-organ dysfunction syndrome. Focal vasculitis of small vessels may lead to gangrene of digits which may be rarely observed in scrub typhus patients. Proper evaluation and early and prompt treatment can lead to effective control of its dreadful complication like gangrene and salvage amputation.

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An Unusual Case of Cerebral Vein Thrombosis in an Adolescent Male

Rajesh Kumar Meena*, Gurmeet Kaur**, Velmurugan***, Kartik Balankhe***

Abstract

Cerebral venous thrombosis (CVT) is a distinct cerebrovascular disorder that mostly affects young adults. The clinical symptoms vary and may include severe headache (90%), focal lateralising signs (50%), seizures (40%) as well as behavioural symptoms, loss of consciousness. Usually CVT are due to secondary causes (70%) and in 30% cases, it is associated with genetic prothrombotic conditions, such as deficiency of antithrombin III, protein C, or protein S, mutation of factor 'V' or prothrombin genes, resistance to activated protein C and hyperhomocystinaemia. We report a case of a 17-year-old male adolescent with cerebral vein thrombosis due to a rare genetic cause.

Key words: Cerebral vein thrombosis, prothrombotic.

Case report

A 17-year-old male presented with complaints of headache and vomiting followed by one episode of partial seizures involving left upper limb and lower limb. There was no preceding aura, loss of consciousness, or focal neurological deficit. He is not a known case of diabetes, heart disease, chronic kidney disease, hypertension, chronic lung disease, seizure disorder, and no significant family history or history of any chronic drug intake. All vitals parameters including blood pressure and physical examination were absolutely normal. CNS examination, gait, tone, power were normal. No cranial nerve abnormality was noted.

Blood investigations including complete haemogram, serum electrolytes, liver and renal functions were normal. Chronic infection disease (e.g., tuberculosis, HIV, HBV and HCV) work-up was not significant. Coagulation profile of this patient is given below.

Coagulation profile of the patient

Test	Test result	Normal range
PT-INR	Normal	
Anti thrombin-III	114	80 - 120
Protein S level	≤ 12.5	60 - 150%
Protein C level	82.89%	70 - 140%
Factor V Leiden	No mutation	Normal pattern
Lupus anti coagulation	Not detected	
Homocysteine	8.59 µmol/l	5.46 - 16.20
Cardiolipin AB-IgG	1.26 GPL/ml	≤ 12 negative
IgM	0.52 MPL/ml	12 - 18 equivocale
IgA	1.99A PL/ml	≥ 18 positive

Imaging studies showed haemorrhagic infarct involving the right parietal region (Fig. 1) secondary to superficial cortical vein thrombosis ((Fig. 2).
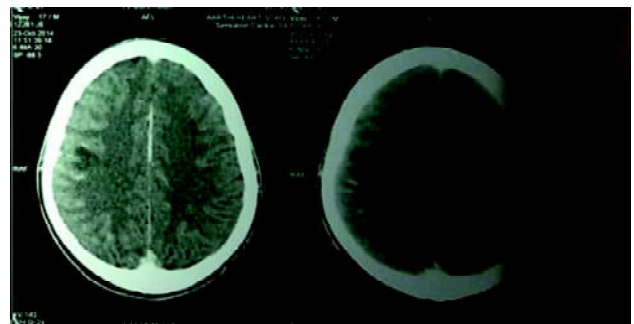
Fig. 1: *Focal hypodensity (oedema) in the right parietal region in post-central gyrus causing mass effect in the form of sulcal effacement with leptomeningeal enhancement.*

Fig. 2: *T2/flair hyper intensity noted in right parietal region which shows blooming gradient suggestive of haemorrhage.*

*Assistant Professor, **Associate Professor, ***Post-Graduate, Department of Internal Medicine, ABVIMS and Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 100 001.
Corresponding Author: Dr Rajesh Kumar Meena, Assistant Professor, Department of Internal Medicine, Department of Internal Medicine, ABVIMS and Dr Ram Manohar Lohia Hospital, Baba Kharak Singh Marg, New Delhi - 100 001. Phone: 9911795612, E-mail: rajesh.marga@gmail.com.
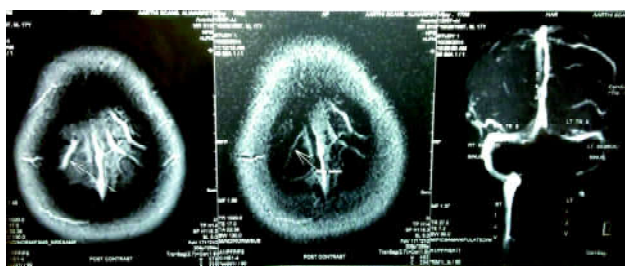
Fig. 3: *Contrast-enhanced MR venogram shows filling defect in the superficial cortical vein (vein of Tolard).*

The patient was initially stabilised with antiepileptic drug, mannitol, and other supportive management. Later on, after the imaging studies, patient was put on low molecular weight heparin and then warfarin. Patient was discharged on oral anticoagulant with proper counselling regarding disease nature, periodic monitoring and treatment details and advised for regular follow-up and screening of the family members for coagulopathy. But due to economic constrains, screening of the other family members had not been done. The patient did not have further symptoms until recent follow-up.

Disscusion

Cerebral venous thrombosis (CVT) is an uncommon, serious disorder due to thrombosis of cortical veins and draining venous sinus. Clinical manifestations can include headache, papilloedema, visual loss, focal or generalised seizures, focal neurologic deficits, confusion, altered consciousness, and coma[1]. Cerebral venous thrombosis is more common in women than men (female to male ratio 3:1). The estimated annual incidence is 3 to 4 cases per million, with 75 per cent of cases occuring in women[2]. This imbalance may be due to the increased risk of CVT associated with pregnancy and puerperium and with oral contraceptives. The incidence of cerebral venous thrombosis in children is at least 0.67 per 1,00,000 children per year[3].

Cause of cortical vein thrombosis including genetic (e.g., protein C, protein S deficiency, anti thrombin, and factor V Leiden mutation deficiency) and acquired disorders (e.g., dehydration, infection, malignancy, drugs, heart disease, head injury, anti-phospholipid syndrome, nephrotic syndrome, and acute gastroenteritis). Prothrombotic conditions are either genetic or acquired[4].

Pathogenesis: The pathogenesis of CVT remains incompletely understood because of the high variability in the anatomy of the venous system . Thrombosis of cerebral veins or dural sinus leads to cerebral parenchymal lesions or dysfunction and occlusion of dural sinus, resulting in decreased cerebrospinal fluid (CSF) absorption, and

elevated intracranial pressure. Obstruction of the venous structures results in increased venous pressure, decreased capillary perfusion pressure, and increased cerebral blood volume. Dilatation of cerebral veins and recruitment of collateral pathways play an important role in CVT.

Protein S deficiency is a rare cause of CVT. Protein S - a vitamin K-dependent glycoprotein, is a co-factor of the protein C system. It is synthesised by both hepatocytes and megakaryocytes and circulates in two forms: 40 to 50 per cent as the free form and the remainder bound to the complement component; only the free form has activated protein C co-factor activity. Protein S deficiency may be genetic or acquired[5].

Genetics of protein S deficiency: Two homologous genes for PS map to chromosome 3. PROS1 (active gene) and PROS2. It is inherited predominantly as an autosomal dominant trait and heterozygous individuals in these families frequently had recurrent thromboembolism. Severe thrombotic complications, including neonatal purpura fulminans, occur in the rare newborn with very low protein S levels, which is occasionally due to homozygous deficiency.

Three phenotypes of PS deficiency have been defined on the basis of total PS concentrations, free PS concentrations, and activated protein C co-factor activity.

Type I: The classic type of protein S deficiency is associated with approximately 50 per cent of the normal total S antigen level, and more marked reductions in free protein S antigen and protein S functional activity (i.e., a quantitative defect).

Type II: Type II protein S deficiency is characterised by normal total and free protein S levels, but diminished protein S functional activity (i.e., a qualitative defect).

Type III: Type III deficiency is characterised by total protein S antigen measurements in the normal range and selectively reduced levels of free protein S and protein S functional activity to less than approximately 40 per cent of normal.

In a patient, phenotypes are described as quantitative (types I or III) or qualitative (type II).

Acquired protein S deficiency: Acquired protein S deficiency occurs during pregnancy and is associated with use of oral contraceptives. Protein S levels may be low in some disorders like disseminated intravascular coagulation and acute thromboembolic disease, HIV infection, nephrotic syndrome[6].

Clinical manifestation

The clinical presentation of patients with heterozygous protein S deficiency are deep venous thrombosis, superficial thrombophlebitis, arterial thrombosis or pulmonary embolism. The mean age is 25 years with the range being 15 to 60 year.

Diagnosis

Levels of total or free PS antigen < 60 to 65 International units/dl are considered to be in the deficient range. Erroneous diagnoses can be made due to the influence of acute thrombosis, co-morbid illness, during pregnancy, and in association with the use of oral contraceptives.

Treatment

The main goals is achieved anticoagulation, using either low molecular weight heparin (LMWH) or heparin followed by oral anticoagulant. Whenever available, endovascular thrombolysis is another option, but its use is typically restricted to patients with a poor prognosis who have not responded to anticoagulation. Antiplatelet drugs may be used as alternatives when anticoagulants are contraindicated.

In a case of life-threatening first thrombotic event or unusual CVT (cerebral and mesenteric vein) most experts recommend lifelong warfarin.

Conclusion

Protien S deficiency is a rare cause of cortical vein thrombosis. Hereditary protein S deficiency is an autosomal dominant trait. Isolated cortical vein thrombosis involving vein of Trolard without involving the deep venous system is an unusual presention of CVT. This case is to highlight one of the unusual presentations of CVT with a rare genetic cause.

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