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E-mail: customer.rfp@rfppl.co.in, customer.rfp@gmail.com

Original Article

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Author Affiliation:
*Department of Nephrology, St. Johns Medical College, Bangalore.

Reprint Request: Limesh M., Assistant Professor, Department of Nephrology, St.Johns medical college,Bangalore – 560034 Karnataka.
E-mail: limijay_2007@yahoo.co.in

Correlation between Limited Sampling Strategy for the Estimation of Mycophenolic Acid Area Under the Time Concentration Curve with Incidence of Rejection and Opportunistic Infections in Post Renal Transplant Patients

Limesh M.*, Atul Desai*, Renuka S.*

Abstract

Mycophenolate mofetil (MMF) has become the single most used immunosuppressant in solid-organ transplantation. Hence this study was done to assess Mycophenolate Mofetil dosing and MPA levels with incidence of acute rejection and opportunistic infections in early post renal transplant period .Forty four consecutive early post transplant patients were randomized 1: 1 to receive concentration controlled (CC) {n=22} or fixed dose (FD){n=22}of mycophenolate mofetil. In CC group 13 patients (60%) were below therapeutic range. 9(40%) were in the therapeutic range and no patients were above the therapeutic range. In FD group 7(30%) patients were below the therapeutic range and 14 (64%) patients were in the therapeutic range. Acute rejection in first 6 months post transplant in CC group was 18% and in FD group was 14% ($p = 0.5$). All rejections were seen within first 3 months post transplant. The overall cumulative incidence of infection in CC group was 24% compared to 7.5% in FD group which reached statistical significance ($p<0.01$) in particular at 3 months post transplant. This study has demonstrates that MMF dose individualization with therapeutic drug monitoring is not an effective method in renal transplant patients to prevent the complications in the post-transplant period such as rejection and infection.

Keywords: MPA Levels; Post Transplant; Infection; Rejection.

Introduction

The success of solid organ transplantation lies in the appropriate utilization of immunosuppressive medications [1]. In simplest terms one would like to administer an adequate dosage of an agent (a dose that adequately suppresses the alloimmune response) while at the same time avoiding toxicity related to excessive immunosuppression or concentration-related secondary toxicities.

Mycophenolate mofetil (MMF) has become the single most used immunosuppressant in solid-organ transplantation. Despite a well-documented relationship and efficacy (in terms of acute rejection prophylaxis) and exposure to mycophenolic acids (MPA) as measured by area under the curve (AUC), excellent results have been achieved using a fixed-dosage regimen.A number of pharmacokinetic studies have shown an increased risk for acute rejection in patients with lower MPA exposure, suggesting that efficacy may improve by adjusting the dose on the basis of plasma concentrations. On the basis of these studies, a target window has been adopted for MPA exposure (area-under-the curve [AUC] values between 30 and 60 mg/L). Accumulating evidence suggests that this target is not reached in every patient with the standard MMF dose, with some studies reporting a 10-fold between-patient variability of MPA exposure, changes of

exposure over time with a fixed MMF dose, and influence of co-medication. Consequently, individualization of the MMF dose may be necessary to achieve adequate MPA exposure in every patient. The risk of post transplant infection is associated with overall degree of immunosuppression, & MPA exposure may have a significant influence on this.

Hence, this study is aimed at studying Correlation between Limited sampling strategy for the estimation mycophenolic acid area under the time concentration curve with incidence of rejection and opportunistic infections in post renal transplant patients.

Subjects and Methods

It was a prospective, exploratory, observational study conducted between June / 2013 to March / 15 in the department of Nephrology, St. Johns Medical College and Hospital, Bangalore.

A total of 44 patients, aged 18 to 62 years, who had received a first or second live related kidney transplant were eligible for inclusion in to the study. Important exclusion criteria were previous graft loss within 12 months after transplantation, multi organ recipient, cardiac death donor, ABO-incompatible transplant, current panel-reactive antibody level >20%. The aim of the study was emphasized on short term outcome of graft (i.e 6 months period) and long term outcome of graft has not been included. Also there were number of drop outs in the initial period and the approval from ethical board and hospital (for waving off the expenses) was limited to sample size 40. The sample size for this pharmacokinetic study was chosen with respect to the exploratory nature of this study and was not based on statistical power considerations.

Immunosuppressive Protocol

Patients were randomized 1: 1 to receive concentration controlled (CC) {n = 22} or fixed dose (FD){n=22}of mycophenolate mofetil, along with 0.1mg / kg / day in 2 divided doses of tacrolimus and 1gram iv od of injection methylprednislone for 3 days, followed by 20mg / day of oral prednisolone. Tacrolimus trough levels were done on day 4 and day 30 of post transplant period and the dose was adjusted to achieve a target trough level of 9-12ng / ml for the first 3 months and 5-7 ng / ml thereafter in both the groups .In both the groups MMF was started as standard dose of 1000mg / d for < 70 kg and 1500mg / d for >70 kg patients in 2 divided doses. In CC group, MMF dose adjustments were made based on five – point limited sampling strategy, namely samplings at 0,0.5, 1.0, 1.5 and 3 hours post dose at day 30 post transplant. In CC group, MMF dose adjustments were done so as to reach MPA AUC closer to 30 – 60 mg / h / L but no dose adjustments were made in FD group even after five – point limited sampling.

Indications for dose reduction or cessation were leucopenia (total white cell count below 4.0 —ÿ 109 / L), persistent anemia (hemoglobin less than 10 g / dL), sepsis requiring hospitalization or persistent diarrhea (greater than 2 weeks duration) in the absence of a defined alternative etiology .Patients were treated with either basiliximab (20 mg on days 0 and 4 after transplantation) or ATG (1 mg / kg on alternate days for 5-7 doses) depending upon the discretion of the treating nephrologists (few nephrologists favor basiliximab over ATG and others vice versa). Also ATG was favoured over basiliximab in patients with historic positive PRA's and second transplant. In CC group ,2 patients received ATG and 6 patients received basiliximab. In FC group, 3 patients received ATG and 5 received basiliximab.

The study was approved by the institutional ethical committee. All patients gave written informed consent. Some of the commonly prescribed comedications included antihypertensives, antivirals, hypolipidemic drugs, proton pump inhibitors, anticoagulants, and vitamins.

It was mandatory that all patients had at least 2 full days of the same MMF dose given twice a day before pharmacokinetic investigation. Patients in CC and FD group had fastened from the previous night and arrived at the Renal unit at 8:00 AM on the day of the test. As per standard protocol, food was allowed only 2 hours after the MMF dosing. Water was allowed as and when required. After a cannula was inserted, blood samples were collected into K2 ethylenediamine tetra acetic acid containing tubes before MMF was administered and at 0, 0.5, 1.0, 1.5 and 3 hours post dose. Altogether, 240 plasma samples were analyzed for 5 point MPA trough levels.

Measurement of Total and Free MPA and MPAG

Plasma concentrations of MPA and MPAG were determined by reverse-phase HPLC, using a Symmetry-C18 column. Briefly, 200 microlit of ethylenediaminetetra-acetic acid plasma was mixed with 100 microlit of acetonitrile containing the carboxy butoxy ether of MPA (15 mg / L) as internal standard. This was followed by sequential addition of 20 microlit of perchloric acid (150 g / L) and 20 pA of sodium tungstate solution (250 g / L).

After mixing and centrifugation, 50 microlit of supernatant was applied to the C-18 column. The mobile phase for elution of the column consisted of solution A (250 ml of acetonitrile and 750 ml of 20 mM phosphate buffer, pH 3.0). and solution B (700 ml of acetonitrile and 300 ml of 20 mM phosphate buffer, pH 6.5), which formed the following

Gradient: 0 to 4.5 mm 3% B; 5 to 12 mm 30% B; 12.5 to 14.5 mm 100% B. Compounds were quantified in parallel by absorbance at 254 and 215 nm. For calibration of MPA and MPAG, drug-free plasma was spiked with either of the two compounds at concentrations of 3 and 200 mg/L, respectively. Using drug-free plasma spiked with MPA or MPAG, the method was found to be linear up to 50 mg/L for MPA and 500 mg/L for MPAG.

The detection limit (signal-to-noise ratio of 3) at 215 nm for plasma samples was 0.01mg/L for total MPA and 0.03 mg/L for MPAG. Between-run imprecision ranged from 3.3 to 9.2% for MPA and 4.1to 6.1% for MPAG. The Centrifree Micropartition System was used to obtain an ultrafiltrate for free MPA determination. For the ultrafiltration procedure, 300 microlit of plasma was added to the sample reservoir and the tube was centrifuged at 2000 x *g* (20 degree C) for 40 min, yielding approximately 150 microlit of ultrafiltrate. This was mixed with internal standard (2.5 mg/L) at a ratio of 10: 1 (vol / vol), and 100 ýd was then injected directly into the C-18 column. A solution of 9 g/L NaC1 adjusted to pH 7.4 with phosphate buffer (67 mmol/L) and spiked with 0.05 mg/L MPA was used for calibration of free MPA determination. The detection limit for free MPA at 215 nm was 0.005 mg/L. Because of an imprecision of >20% at *0.005* mg/L, the limit of quantification for free MPA was set at 0.01 mg/L. The within-day imprecision ranged from 6.5 to 1 1.8% and the between-day imprecision from 7.2 to 15.8%. Before starting this investigation, it was confirmed that freezing and thawing of samples did not influence the protein binding of MPA.

Pharmacokinetic Analysis

The following pharmacokinetic data for MPA, free MPA, and MPAG were determined: time to maximum concentration *(Tmax***),** maximum concentration *(Cmax* mg/L), area under the curve (AUC) from 0 to 2 h (mg X h/L) using the linear trapezoidal rule, and minimum concentration (C*min* ,mg/L). *C min* was defined by the formula: *Cmin* =(C*time* 0 +*Ctjme* 2) / 2. The pharmacokinetic analysis was performed using the computer program BiAS .

Acute Rejection Episodes and Infection Episodes

The primary outcome criterion for the determination of the PK relationship for MPA was the occurrence of acute rejection episodes and infection rates over the 6-mo study period after transplantation. Seven of 44 patients experienced at least one acute rejection episode during the 6-mo study period, none of them had two rejection episodes.

Acute rejection episodes were diagnosed based on graft biopsy, histological examination and classification of a core biopsy was done according to the Banff criteria . If a biopsy was logistically impossible or clinically contraindicated, the diagnosis of "presumed rejection" was based on clinical judgment (supported by one or more of the following clinical findings: increased body temperature, graft swelling, graft tenderness, rise in serum creatinine level of more than 20% from the baseline level, or oliguria).

Acute rejection episodes were treated initially with high-dose intravenous corticosteroids (1 g/day of inj methylprednisolone, for 3 consecutive days). If the rejection episode failed to respond to this therapy, treatment with antithymocyte globulin was started, but none of the patients required ATG for reversal of rejection. Bacterial infection was defined as fever with positive identification of an organism on culture, or fever with accompanying clinical features of bacterial infection including neutrophilia and an elevation in C-reactive protein, rapidly improving with antibiotics; Viral infection was defined as Clinical features of viral infection, with either (a) viral identification on histology, polymerase chain reaction, culture or electron microscopy or (b) leucopenia or raised alanine transaminase, with symptoms resolving following anti-viral therapy.

Other Clinical Variables in the Multivariate Analysis

Other donor and recipient variables with a potential impact on clinical endpoints were analyzed: donor and recipient demographics (age, ethnicity, gender, primary disease, time on dialysis, donor source and, CMV serology, HLAmismatch and panel reactive antibody sensitization, repeat transplantation);total daily MMF dose (individual MMF dose multiplied by dosing frequency); delayed graft function (dialysis requirement in the first week post transplantation).

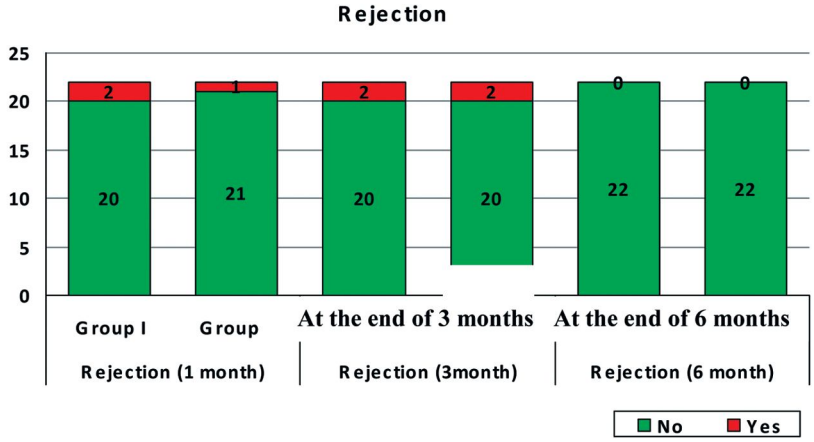
Statistical Analysis

The data was analysed using Statistical Package for the Social Sciences (SPSS17 for Windows (SPSS

Inc., Chicago, IL). The comparison of outcome amongst the two study groups was done by Chi-square test and paired student t test. For comparisons of continuous parameters between groups and within a group over time, repeated measures ANOVA was used. The association of MPA levels and other clinical variables with continuously distributed data was analyzed by population averaged linear regression, using robust standard errors. Data showing a skewed distribution underwent logarithmic transformation. A p value of <0.05 was considered significant.

Results

This was a prospective, exploratory, observational study conducted between June 2013 and March 2015, in the department of Nephrology, St. Johns Medical College and Hospital, Bangalore.



The characteristics of study population is shown in figure 1, a total of 44 patients who underwent renal transplantation were included in the study. There were a total of 22 (50%) patients who received concentration controlled mycophenolate triple immunosuppressive therapy (CC group) and 22 (50%) patients who received fixed dose mycophenolate triple immunosuppressive therapy (FD group).

Most of the patients (47.5%) were in the age group 40 – 50 years. In the population studied 13 patients were females and 31 patients were males.

Table 1: Charlsons index (n = 44)

	Charlsons index		Total	p value	Significance
	0	1			
CC Group	20	2	22	1	Not Significant
FD Group	19	3	22		
Total	39	5	44		

Table 2: Induction regimen (n = 44)

	Induction Regimen		Total	p Value	Significance
	Nil	Yes			
CC Group	14	8	22	0.517	Not Significant
FD Group	16	6	22		
Total	30	14	44		

Table 3: Difference in blood urea during follow up (n = 44)

	Group	n	Mean	SD	p value	Significance
Blood Urea (1month)	CC Group	22	29.82	12.06	0.054	Not Significant
	FD Group	22	36.68	10.82		
Blood Urea (3month)	CC Group	22	31.55	10.65	0.155	Not Significant
	FD Group	22	36.14	10.39		
Blood Urea (6month)	CC Group	22	34.77	12.47	0.858	Not Significant
	FD Group	22	35.41	10.97		

Table 1 shows that the difference in Charlsons index between CC group and FD group is not statistically significant, hence both groups are comparable.

Table 2 shows that the difference in usage of induction regimen between CC group and FD group is not statistically significant, hence both groups were comparable.

Table 3 shows the mean difference in blood urea between CC group and FD group at 1, 3 and 6 months. Even though the mean blood urea was higher in FD group at 1, 3 and 6 months, the difference was not statistically significant.

The mean creatinine of FD group was comparatively higher at 1, 3 and 6 months when compared to CC group . But the difference between the groups was not statistically significant (Table 4).

The serum albumin at 1, 3 and 6 months was almost similar in both the groups (Table 5).

Table 4: Difference in creatinine during follow up (n = 44)

	Group	n	Mean	SD	p value	Significance
Creatinine (1month)	CC Group	22	1.064	0.228	0.064	Not Significant
	FD Group	22	1.223	0.319		
Creatinine (3month)	CC Group	22	1.11	0.25	0.181	Not Significant
	FD Group	22	1.24	0.36		
Creatinine (6month)	CC Group	22	1.173	0.278	0.402	Not Significant
	FD Group	22	1.255	0.358		

Table 5: Difference in serum albumin during follow up (n = 44)

	Group	n	Mean	SD	p value	Significance
Albumin (1month)	CC Group	22	3.936	0.376	0.764	Not Significant
	FD Group	22	3.905	0.32		
Albumin (3month)	CC Group	22	3.986	0.381	0.713	Not Significant
	FD Group	22	3.95	0.26		
Albumin (6month)	CC Group	22	3.973	0.299	0.91	Not Significant
	FD Group	22	3.964	0.228		

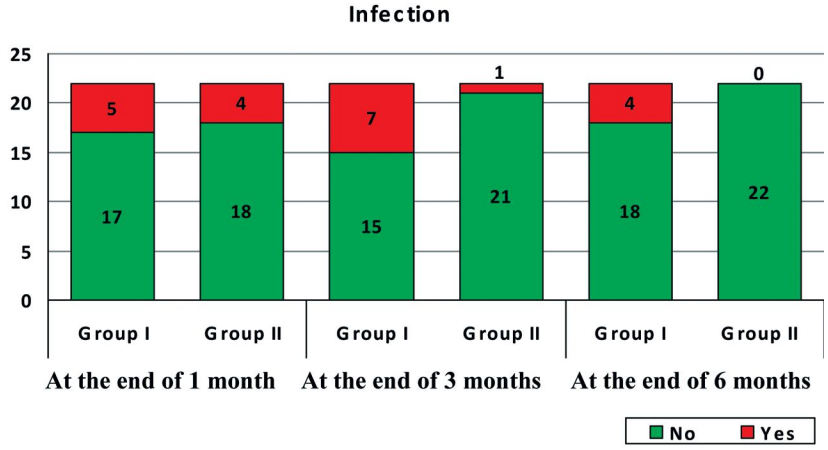


Fig. 2: Incidence of rejection during follow up (n=44)

Table 6: Incidence of rejection during follow up

	Rejection (1month)		Rejection (3month)		Rejection (6 month)	
	No	Yes	No	Yes	No	Yes
CC Group	20	2	20	2	22	0
FD Group	21	1	20	2	22	0
p value	0.5		0.697		Not applicable	
Result	Not Significant		Not Significant			

Acute rejection in first 3 months post transplant in CC group was 18% (4/22) and in FD group was 14% (3/22), and did not reach statistical significance (p=0.5) (Figure 2 and Table 6). All rejections were seen within predominantly first 3 months post transplant period.

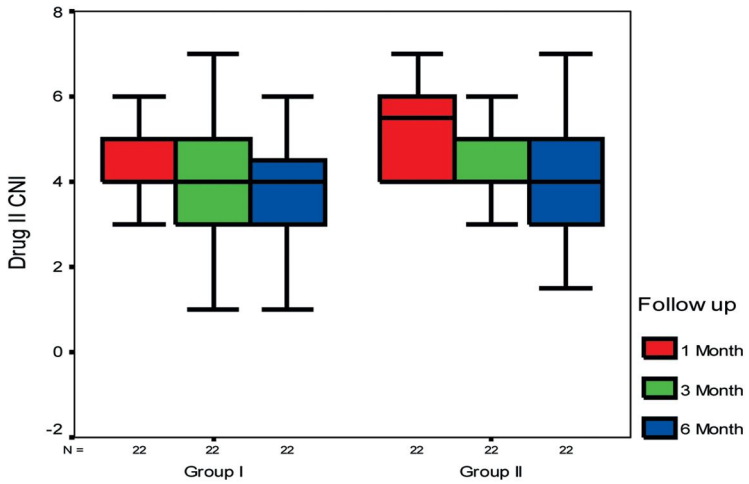


Fig. 3: Incidence of infection during follow up (n=44)

Table 7: Incidence of infection during follow up

	Infection (1month)					Total	p value	Result
	UTI	**Nil**	**Pneumonia**	**Pyelonephritis**	**Sepsis**			
CC Group	3	17	1	1		22	0.5	Not Significant
FD Group	2	18			2	22		
	4	35	1	1	2	44		
			Infection (3month)			Total	p value	Result
	UTI	Nil	CMV	TB	Sepsis			
CC Group	3	15	2	1	1	22	0.023	Significant
FD Group		21	1			22		
	3	36	3	1	1	44		
			Infection (6month)			Total	p value	Result
	UTI	Nil	CMV	TB	Pneumonia			
CC Group	1	18	1	1	1	22	0.054	Not Significant
FD Group		22				22		
	1	40	1	1	1	44		

Infections which were seen during follow up are urinary tract infection, pyelonephritis , pneumonia, tuberculosis, cytomegalovirus infection and sepsis.

5 patients in CC group and 4 patients in FD group had developed infection by the end of first month of follow up. 7 patients in CC group and 1 patient in FD group had developed infection by the end of third month of follow up. 4 patients in CC group and no patients in FD group had developed infection by the end of sixth month of follow up. Overall incidence of infection was much higher in CC group compared to FD group, but this difference was statistically significant at 3 months. The overall cumulative incidence of infection in CC group was 24% compared to 7.5% in FD group which reached statistical significance ($p<0.05$). In CC group, almost all patients who developed infection had MMF trough levels within the therapeutic range i.e., 30-60 mg/h/L, except for 3 patients who developed infection below the therapeutic range which included 2 patients with UTI and 1 patient with CMV infection. Similarly, in FD group 3 patients developed infections within therapeutic range, 1 patient above and below therapeutic range.

The difference in tacrolimus dose between CC group and FD group was not statistically significant (Figure 4).

The mean MMF dose was 1.368 in CC group and 1.182 in FD group, this difference is not statistically significant. However the difference in MMF dosing at 3 months and 6 months, between CC group and FD group were statistically significant.

The mean steroid dosing in CC group was 20.23, 17.73 and 17.05 at 1, 3 and 6 months respectively. The mean steroid dosing in FD group was 19.77, 17.5 and 16.82 at 1, 3 and 6 months respectively. This difference is not statistically significant.

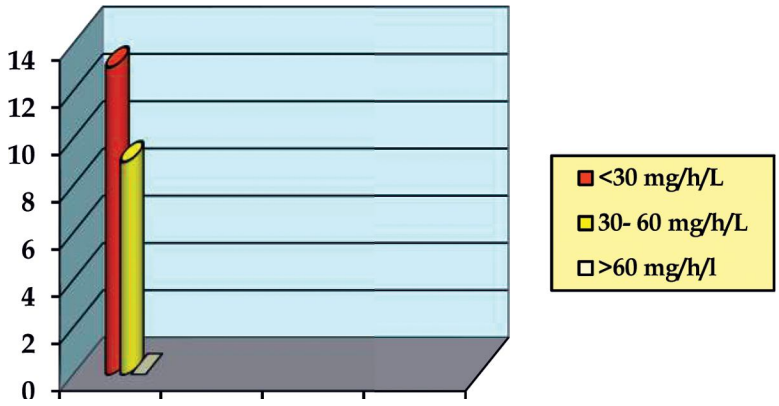


Fig. 4: Tacrolimus dose (n=44)

Table 8: Difference in MMF dosing (n = 44)

	Group	n	Mean(gms/d)	SD	p value	Significance
MMF (1month)	CC Group	22	1.068	0.234	0.124	Not Significant
	FD Group	22	1.182	0.246		
MMF (3month)	CC Group	22	1.386	0.435	0.021	Significant
	FD Group	22	1.136	0.228		
MMF (6month)	CC Group	22	1.295	0.295	0.025	Significant
	FD Group	22	1.136	0.228		

Table 9: Difference in steroid dosing (n = 44)

	Group	n	Mean(g/d)	SD	p value	Significance
Steroid (1month)	CC Group	22	20.23	3.93	0.703	Not Significant
	FD Group	22	19.77	3.93		
Steroid (3month)	CC Group	22	17.73	4.56	0.866	Not Significant
	FD Group	22	17.5	4.3		
Steroid (6month)	CC Group	22	17.05	4.98	0.878	Not Significant
	FD Group	22	16.82	4.77		

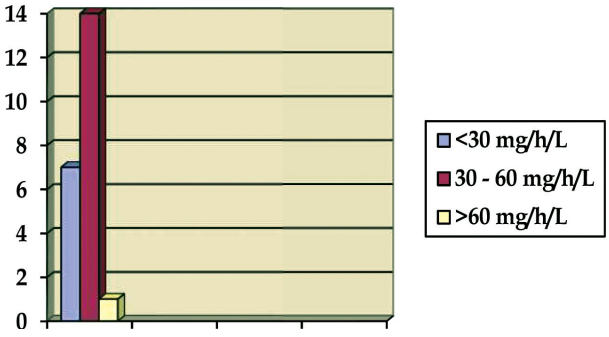


Fig. 5: MMF trough level (n=22) at 1 month post transplant period

As shown in Figure 5, MMF trough level of < 30 mg/h/L was observed in 13 patients, therapeutic range of 30 – 60 mg/h/L was observed in 9 patients and no patients had MMF trough level above 60 mg/h/l in CC group at 1 month post transplant period. Accordingly , only 10 patients needed an increase in the dose of MMF, as other 3 patients could not tolerate(GI symptoms) the hiked dose and 9 patients in therapeutic range were continued on same dose of immunosuppression in CC group.

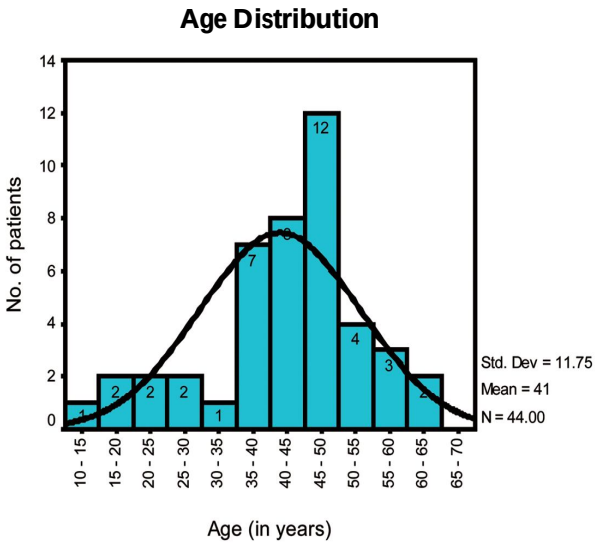


Fig. 6: MMF trough level (n=22) at 1 month post transplant period

Figure 6 shows, in FD group, 14 patients were within the therapeutic range (30-60 mg/h/l) and 7 patients were below therapeutic range. However, no dose adjustments were made in this group.

Discussion

In this study , maximum number of transplants were in the age group of 35-55 years and included 70% male recipients . In terms of induction regimen, 2 patients in CC group and 3 patients in FD group received ATG which did not reach statistical significance, and similarly for basiliximab in both the groups (p=0.8). Only 5 patients (3 in FD & 2 in CC group) underwent second transplant. Native kidney disease in both groups included predominantly chronic glomerulonephritis, diabetic nephropathy and chronic interstitial nephritis on multivariate analysis.The degree of HLA mismatch did not reach statistical significance (p=0.5). The dosing of Tacrolimus (p value=0.9) and steroids (p value=0.8) in both the groups also did not reach statistical significance(in both the groups). The mean MMF dose was 1.368g/d in CC group and 1.182g/d in FD group, but the difference in MMF dosing at 3 months and 6 months, between CC group and FD group was statistically significant., CC group receiving more dose than FD group. All patients were followed for a minimum of 6months; median of 7 months (range : 7-9 months).

There were no deaths or graft loss occurred during this period. Blood urea, serum creatinine and albumin were almost similar in both groups. None of the patients had delayed graft functions.

In CC group 13 patients (60 %) were below therapeutic range .Only 10 patients in CC group needed an increase in dose (as 3 could not tolerate the hiked dose due to GI intolerance and hence were continued on same dose). None of the patients were above the therapeutic range. In FD group only 7 patients (30%) were below the therapeutic range and 14 patients (64 %) were within the therapeutic range. Acute rejection in first 6 months post transplant in CC group was 18% and in FD group was 14% (p=0.5). All rejections were seen within first 3 months post transplant. The overall cumulative incidence of infection in CC group was 24% compared to 7.5% in FD group which reached statistical significance (p<0.01) in particular at 3 months post transplant.

The majority of patients in the CC group regimen did not reach MPA therapeutic levels day 30 after transplantation .An intensified dosing regimen may also have potential drawbacks .It increases the risk for overexposure and toxicity if the starting dosage is either too high or given for too long.

CC group regimen was generally well tolerated during the higher dosage phases, and the majority of patients stayed on the intensified dosing scheme. Previous studies that used higher MMF dosages in combination with CsA [2-6] or standard dosages in combination with tacrolimus [7,8] reported similar MPA exposure (<30 mg/h per L).

Mean MPA-AUC in this study was 29.4 mg/h per L on day 30 in CC group and 26.7 mg/h per L on day 30 in FD group.

Mean MPA-AUC was only 33.7 mg/h per L on day 14 in the Apomygre study, and levels <40 mg/h per L were not achieved until month 1 using a concentration-controlled approach [9]. Similarly, mean MPA-AUC of CsA treated patients on day 10 was 34.4 mg/h per L in the Fixed-Dose Concentration-Controlled (FDCC) Study [10] .

There was a large interindividual variation of PK data, despite the fact that all patients were receiving the same per kg body weight –adjusted MMF dosage.

Both the group were comparable either in terms of native kidney disease, induction regime ,HLA matching ,immunosuppressive protocol. There were no difference in cumulative incidence for rejection in both the groups, however there was increased incidence of infection noted in the CC group. In FD group, majority of the patients were in the therapeutic range, compared to CC group. Hence, it can be concluded that there are no added benefits of monitoring MPA levels in post transplant period Our data has demonstrated that therapeutic drug monitoring of MMF doses has no role in improving clinical outcomes in post transplant period.

Like the FDCC (FD vs. CC) MMF trial [11], which showed no improvements in outcomes with CC MMF dosing, our study also showed similar results.

Recommended therapeutic window for MPA AUC has been derived from the original study done by Binu S et. al.[12], in renal patients who were on triple therapy with MMF, prednisolone and tacrolimus.

The variability in MPA exposure following the administration of MMF observed in this study and previously reported by other investigators is a result of its complex pharmacokinetics. In the early posttransplant period, MPA AUC in renal allograft recipients is positively predicted by levels of serum creatinine and serum albumin [13], reflecting the impact of renal function and protein binding on MPA clearance.

In the current study, patients in the CC group received almost similar doses of MMF as the FD group at 1 month,but at 3 month the CC group had received increased dose with an increased occurrence of infections. A possible explanation may be that there were insufficient numbers of patients to identify

significant differences between groups in adverse events. Other studies attempting to correlate MPA exposure with adverse events have also yielded inconsistent findings; however, one study reported a correlation between adverse events and MPA AUC and C30 (30-min post-dose) MPA levels [14] , while others found a relationship between free MPA levels and hematological toxicity [15,16]. The lack of consistent correlations between MPA levels and adverse events may reflect the nature of the events, which have multiple causes, and may be further complicated by the fact that small numbers of patients were evaluated in many of these studies as well.

MPA monitoring is not yet widely accepted due to the complexities of MPA pharmacokinetics, lack of accurate measurement tools and MPA AUC calculations.

The concentration–effect relationship for mycophenolic acid (MPA), and the high variability in MPA concentrations in patients on standard dose mycophenolate mofetil (MMF) therapy, for some centers has provided enough evidence to implement therapeutic drug monitoring (TDM) for MMF in daily practice. Two randomized trials, Adaption de Posologie du MMF en Greffe Renale (APOMYGRE) [18] and fixed-dose versus concentration controlled (FDCC) [17] investigated the added benefit of TDM for MMF in renal transplant recipients.

The APOMYGRE study showed a significant reduction in the incidence of acute rejection in concentration controlled patients, while the FDCC study had a negative outcome, despite a similar study design. In Opticept, concentration-controlled MMF combined with reduced level calcineurin inhibitor was found to be noninferior to concentration-controlled MMF combined with standard level calcineurin inhibitor and noninferior to fixed-dose MMF combined with standard level calcineurin inhibitor.

There are few limitations in the study 1) Sample size need to be larger to power the study. 2) 5-point MPA trough levels were estimated once only (30 days post transplant) in both the groups, even after dose adjusting in CC group. Many studies have looked at sampling at various intervals, which was not feasible in our study due to financial constraints and patients co -operation for drawing multiple blood samples

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Original Article

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Clinical Study and Management of Bladder Outlet Obstruction in Adult Men

Author Affiliation:
*Department of Urology,
**Department of Radiology,
Deccan College of Medical Sciences & Owaisi hospital, Hyderabad, Andhra Pradesh, India.

Reprint Request:
Mohammad Amjad Khan, Dept of Urology, Deccan College of Medical Sciences & Owaisi hospital, Hyderabad, Andhra Pradesh, India-500058
E-mail: amjad_786_2000@yahoo.com

Mohammad Amjad Khan*, Amtul Siddiqua, K. Eashwer Goud*, Adil Majeed*, G.S.N. Murthy**, J. Venkateshwarlu**, Anand Abkari**, Alka Prasad****

Abstract

Introduction: Bladder outlet obstruction is one of the commonest causes of LUTS. Most common cause of BOO in men is BPH. Other common causes are bladder stones and bladder cancer. There is also increased incidence of BOO in younger age group too. In younger age group urethral stricture is commonly seen. *Aims and objectives:* To study various etiology and clinical presentations and management of bladder outlet obstruction according to standard surgical guidelines using either minimally invasive or conventional means. *Materials and Methods:* A total of 100 cases were admitted in Deccan College of Medical Sciences and Owaisi Hospital and Research Centre, Hyderabad, India from 1st September 2011 to 31th August 2013 with objective evidence of bladder outlet obstruction and study was conducted accordingly. *Discussion:* This study is a prospective observational study. The most common cause of BOO was evaluated and was treated according to standard line of treatment and follow up done accordingly. *Conclusion:* Bladder outlet obstruction is a clinical entity of diverse etiology and is a potentially curable illness if diagnosis is made early and treated according to the standard guideline of management. In patients not fit for surgery obstruction can be relieved by catheterization and treatment can be planned once patient is fit for definitive procedure. A further population based study is needed to identify the exact prevalence of BOO in different age groups and effectiveness of available modality and long term complications.

Keywords: Bladder outlet Obstruction; LUTS; BPH; Urethral Stricture.

Introduction

BOO is a common cause of LUTS in men. Most common cause of BOO in men is BPH. Other common causes are bladder stones and bladder cancer. Due to increased life expectancy there is an increased incidence of bladder outlet obstruction. For example, BPH is the most common cause of BOO In men above 70 years. There is also increased incidence of BOO in younger age group too. In younger age group urethral stricture is commonly seen.

Complications of BOO can be devastating. Long term or high grade BOO can permanently damage all parts of the urinary system. Complications include: bladder and kidney stones, kidney failure, recurrent UTI, urinary retention, urinary incontinence. Early diagnosis is important and can often lead to a simple and effective cure.

Numerous gender specific etiologies are responsible for BOO. BOO may be induced by specific functional and anatomical causes. The resulting obstruction frequently produces LUTS. Categorizing and understanding these entities is crucial as specific diagnostic modalities may be used to fully delineate the degree of BOO and any secondary

issues. Although urodynamic evaluation and pressure flow evaluation is the gold standard diagnostic tool, other modalities may also be used including post void residual analysis, urinary flow rate, cystoscopy and selected radiologic ones. Patients self appraisal of symptoms using IPSS is relevant to the initial assessment and subsequent longitudinal follow up.

Aims and Objectives

To study various etiology and clinical presentations and management of bladder outlet obstruction according to standard surgical guidelines using either minimally invasive or conventional means.

Inclusion Criteria

1. Any adult male comes with symptoms suggestive of BOO.
2. Above 18 years age group male patients.
3. Patients visiting first time for the symptoms to our institution.
4. Patient's consent for the thesis.

Exclusion Criteria

1. Patient's refusal for the thesis work.
2. Patients already on treatment for symptoms suggestive of BOO before coming to our institution.
3. Female patients.
4. Male patients below 18 years of age.
5. Patients already taken treatment for BOO in the past.

Materials and Methods

A total of 100 cases were admitted in Deccan College of Medical Sciences and Owaisi Hospital and Research Centre, Hyderabad, India from 1st September 2011 to 31th August 2013 with objective evidence of bladder outlet obstruction. The causes of obstruction as follow BPE (55%), carcinoma of prostate (9%), urethral stricture (15%) (Fig: 1), bladder cancer (8%) (Figure 3) and vesical calculus (13%) (Figure 2).

Ultrasound was the initial modality to diagnose BOO. X-ray KUB, I.V.P. series and CT [1-4] abdomen and pelvis were carried out were ever it was necessary, apart from the routine investigations. Serum PSA and prostatic biopsy was done in cases of suspected carcinoma prostate. Cystoscopy was performed in suspected cases of bladder cancer and biopsy was taken.

In cases of BPH, prostatic size more than 100cc was treated by open prostatectomy [5,6] and less than 100cc either medical management [7] (alpha blockers) or TURP [8], patients with less than 100g prostate, who were unfit for surgery and not willing for surgery, alpha blockers were given. For open prostatectomy [9,10], freyer's procedure was done as described in literature. After 14days suprapubic catheter was clamped and 3 way urethral catheter was removed. If patient had no voiding difficulties, suprapubic catheter was removed after 1day. Most of the patients had no major post operative complications.

Medical management using alpha blockers like tamsulosin 0.4mg HS and combination therapy given to patients. TURP [11] was done using STORZ IGLESIAS resectoscope with 30 degree telescope. 3 way urethral catheters removed after 5days. 5 patients out of 45 complained of poor urinary stream, which gradually improved within 1week and had no surgical intervention. Rest had no complications and followed up regularly.

Early stages of carcinoma prostate were treated with radical prostatectomy. All cases of carcinoma prostate were late stage. In cases of late stage carcinoma prostate (stage III & IV), maximum androgen blockade by orchidectomy using scrotal incision and postoperative anti androgen was given. Those prostate refractory to antiandrogen, chemoradiation [12] was advised. 8 patients responded well to B/L orchidectomy and anti androgen therapy. Only 1 patient had progressive elevation of serum PSA at 3months of follow up following maximum androgen blockade. He was advised chemoradiation.

For cases of bladder cancer with obstruction, radical cystectomy with ileal conduit was done. All 8 cases had uneventful post op.

Cases of bladder stone with size more than 3cms were treated by cystolithotomy and size less than 3cms were removed by cystolithotripsy. Cystolithotomy done using vertical midline incision, bladder closed in two layers using catgut 1-0 and 2-0. Suprapubic catheter removed after one week and urethral catheter two days after suprapubic catheter. In cystolithotripsy cystoscope is used to visualised

stone. Fragmented using lithotripter and removed. Urethral catheter is removed after 2 days.

Cases of stricture urethra managed with VIU. In cases of stricture following urethral rupture, immediate trocar suprapubic catheterization was done to relieve bladder outlet obstruction followed by radiological evaluation and managed by VIU. All cases of stricture were less than 3cms managed by VIU.

Subsequent to the definitive procedure patients were followed up for a periods ranging from 3 to 6 months followed by every yearly. Symptom improvement, physical examination and ultrasound were the main diagnostic tool for follow up. Serum PSA was done every 3months for follow up cases of carcinoma prostate, in addition to ultrasound. In rest of the cases of bladder outlet obstruction follow up done as described above.

Analysis and Results

During this study a total of 100 cases with objective evidence of bladder outlet obstruction were studied and the age distribution is as shown in diagram. The mean age is 57 years with range from 18 years to 90 years. The peak incidence is seen in 7th decade, followed by 8th and 5th decade. Least incidence was seen in 2nd decade (Table 1). The most common cause was BPH followed by stricture urethra, vesical calculus, carcinoma prostate and bladder carcinoma (Table 2). Out of 100 cases of BOO, 45 underwent TURP, 08 cases were treated with medical therapy, 2 cases underwent

Table 1: Age distribution

Age in years	No. of patients
Less than 20	01
20-29	03
30-39	03
40-49	16
50-59	09
60-69	42
70-79	19
80 and above	07

Table 2: Etiology

Etiology	No. of Patients
BPH	55
Carcinoma prostate	09
Bladder carcinoma	08
Bladder stones	13
Urethral stricture	15

Table 3: Management

Management	No. of patients
TURP	45
Medical management of BPH	08
Open prostatectomy	02
B/L Orchidectomy	09
VIU	15
Radical cystectomy and ileal conduit	08
Cystolithotomy	04
cystolithotripsy	9

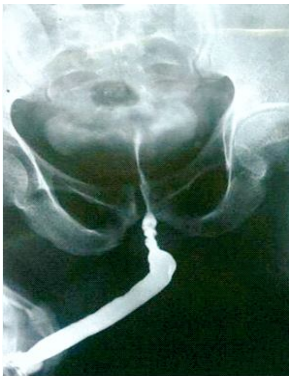

Fig. 1: RGU showing stricture urethra

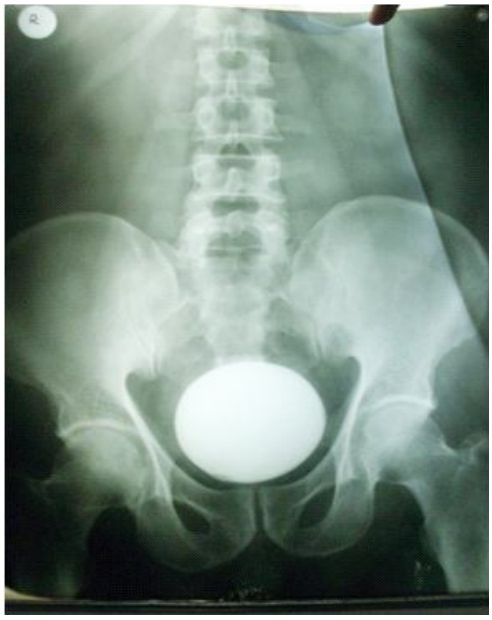

Fig. 2: Vesical calculus

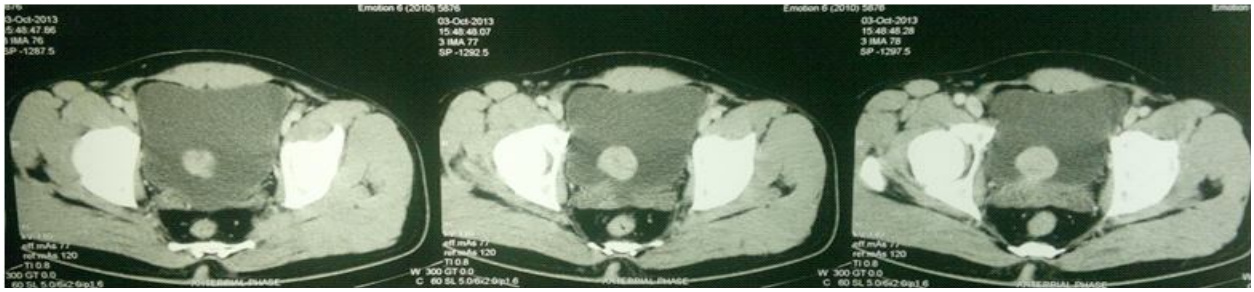

Fig. 3: CECT Abdomen & pelvis showing bladder tumor

open prostatectomy, 9 underwent cystolithotripsy, 9 underwent B / L orchidectomy, 8 underwent radical cystectomy with ileal conduit, 15 underwent VIU, 4 underwent cystolithotomy (Table 3).

Discussion

This study is a prospective observational study in which a total number of 100 cases of BOO were studied. The most common cause of BOO was BPH accounting for more than half the number of cases. Patient who underwent TURP had less morbidity, less complications and short duration of stay compared to open prostatectomy. Patients who were on medical treatment (unfit for surgery and those not willing for surgery) had less symptomatic improvement compared to TURP. Those who opted for surgery had better symptomatic improvement. Of the procedure mentioned above TURP seems to be better accepted by patients of BPH. In this study all cases of carcinoma prostate presented late underwent maximum androgen blockade. This implies significance of screening for early detection of carcinoma prostate. Cases of vesical calculus, those underwent cystolithotripsy had less morbidity and shorter hospital stays compared to those who underwent suprapubic cystolithotomy. Cystolithotripsy considered being better approach than cystolithotomy in treating vasical calculus. Cases of urethral stricture underwent VIU as had short segment of stricture and had good symptomatic improvement. Those who underwent radical cystectomy and ileal conduit for carcinoma bladder had advanced staging of disease but had better disease progression free survival. Few cases of primary bladder hypertrophy and bladder neck stenosis came to our institution for treatment but those were either had taken treatment some were else or they were already on treatment before coming to our institution hence not included in this study.

Conclusion

Bladder outlet obstruction is a clinical entity of

diverse etiology and is a potentially curable illness if diagnosis is made early and treated according to the standard guideline of management. The immediate obstruction can be relieved by catheterization either urethral or suprapubic. In patients not fit for surgery obstruction can be relieved by catheterization and treatment can be planned once patient is fit for definitive procedure. A further population based study is needed to identify the exact prevalence of BOO in different age groups and effectiveness of available modality and long term complications.

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author.rfp@rfppl.co.in

author.rfp@gmail.com

Original Article

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Analysis of Hepcidin, Ferritin, CRP and Iron Levels in ESRD Patients and Their Correlation in CKD-4 & 5 Stages with/without Iron Intake

Rakesh Y.*, Varaprasada Rao K.*, Praveen Kumar Kolla*

Author Affiliation:
*Department of Nephrology, Narayana Medical College & Hospital, Nellore-524003, A.P. India.

Reprint Request:
Praveen Kumar Kolla
Professor & Head, Department of Nephrology, Narayana Medical College Hospital, Nellore-524003, A.P. India.
E-mail: drkolla2002@yahoo.co.in

Abstract

Inflammation interferes with iron utilization in chronic kidney disease through hepcidin. In our study, iron levels, ferritin, CRP and hepcidin levels were analyzed in newly diagnosed end-stage renal disease (ESRD) patients. A total of 50 ESRD patients and 5 healthy controls were studied. 40 recently detected ESRD patients on hemodialysis and 10 patients with Stage 4 CKD not received HD or parenteral iron, 22 out of 40 ESRD patients had already received prior parenteral iron or blood products. The ESRD patients had a significantly lower estimated albumin; and higher transferrin saturation (TSAT) and raised serum ferritin and Hepcidin levels. Hepcidin levels correlated significantly with Ferritin levels. Whereas ferritin levels correlated significantly with CRP levels. There have been elevated serum hepcidin levels in ESRD patients more in those receiving Iron therapy. High hepcidin levels could explain the functional iron deficiency. Larger randomized multicenter studies could throw more light on the diagnostic and therapeutic potentials of using Hepcidin-25 levels in regular practice.

Keywords: Chronic Kidney Disease; Hepcidin; CRP; Hemodialysis.

Introduction

The burden of chronic kidney disease (CKD) is increasing all over the world including in India. The best-known adverse consequence of CKD is end-stage kidney disease (ESRD). The incidence of ESRD in India has been estimated at 165–225 per million population [1].

The anemia that accompanies chronic renal disease (CKD) is associated with precocious mortality and morbidity rates, as well as with a decrease in life quality of patients. The chief etiology of anemia in CKD is erythropoietin (Epo) deficiency. Despite the widespread Epo use, over 50% of the patients do not reach the target hemoglobin levels[1,2]. The most common reason for poor response to Epo therapy is iron deficiency[1]. Inflammation has been implicated as another important cause of poor response[3]. C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) are acute phase reactants that have been used to reliably assess the degree of inflammatory activation [4]. Hepcidin, a regulator of body iron stores, has been identified to play a critical role in the pathogenesis of anemia of chronic disease.

Ferritin, other than being a marker of body iron stores, also increases in acute inflammation, and becomes less valuable as an indicator of iron status during inflammation [5]. However, studies have suggested that parenteral iron therapy might itself contribute to morbidity and mortality by inducing a pro-inflammatory state, due to increased oxidative

stress [6]. Additionally, assessment of iron status itself may be rendered difficult on account of inflammatory activation. A vast majority of Indians are vegetarians, and anemia due to iron deficiency is very common in the general population [7]. Hence, in our study, we analyze the body iron status, levels of CRP and hepcidin levels in ESRD population.

Materials and Methods

A non randomized cross sectional observational study was conducted at Narayana Medical College in the Department of Nephrology from January 2014 to January 2016. Study approved by Institutional ethics committee, a written informed consent was taken from all patients. All patients fulfilling inclusion criteria were screened and investigation done. Recently diagnosed ESRD patients on dialysis and CKD stage 4 of either sex were included. Healthy adult individuals were recruited as controls. The exclusion criteria were: The exclusion criteria were: age less than 18 years, evidence of acute infection or trauma in the last four weeks, history of parenteral iron injection in the last 14 days, history of blood transfusion in the last one month, hemoglobinopathies, malignancy, recent overt blood loss, and post-transplant status. 50 CKD patients including 10 patients with stage 4 CKD and 40 patients with ESRD who had been recently initiated on dialysis (< 3 months) and 5 healthy volunteers as controls.

All patients underwent a thorough physical examination, nutritional status and anthropometrical data, Skin fold thickness, mid arm muscle circumference (MAMC), Body fat percentage and Body mass index.

For dialysis patients, the modality and schedule of dialysis were also recorded. Hemogram, serum iron, total iron binding capacity (TIBC), serum ferritin, percentage transferrin saturation (TSAT), and quantitative CRP levels were analyzed. Anemia patients were stopped to oral iron for a week before sampling.

Serum Iron: Serum iron was measured as recommended by international committee for standardization. Protein was precipitated and chromogen was added to supernatant followed by measurement of absorbance

TIBC: Excess iron was added to sample as ferric chloride, excess unbound iron was removed with magnesium carbonate. The iron concentration was measured.

Serum Ferritin: Ferritin was estimated by an immunometric enzyme immunoassay.

Serum CRP: It was estimated using quantitative CPR assay kit, principle was based on immune precipitation in a liquid phase.

Serum Hepcidin: hepcidin-25 was estimated using the DRG® Hepcidin 25 bioactive ELISA (EIA-5258) hormone enzyme immune assay kit. The DRG Hepcidin-25 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The micro titer wells are coated with a monoclonal (mouse) antibody directed towards an antigenic site of the Hepcidin-25 molecule. Endogenous Hepcidin-25 of a sample competes with a Hepcidin-25-biotin conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off and a streptavidin-peroxidase enzyme complex is added to each well. After incubation, unbound enzyme complex is washed off and substrate solution is added. The blue colour development is stopped after a short incubation time, turning the colour from blue to yellow. The intensity of colour developed is reverse proportional to the concentration of Hepcidin in the sample.

Statistical Analysis

Data were presented as mean ± S.E. ANOVA test was used to test the mean difference between three groups. Pearson correlation test was used to test the correlation between the variables. All the p value of less than 0.05 was considered as statistically significant.

Results

The study includes 55 individuals, 40 recently detected ESRD patients on hemodialysis and 10 patients with Stage 4 CKD not received HD or parenteral iron, 22 out of 40 ESRD patients had already received prior parenteral iron or blood products and 5 healthy control subjects. Males 39 and Females 16 were recorded. The mean age of patients in the study group was 48.76 ± 13.983 years. The minimum age of our individual are 20 years and maximum age was 74 years. The CKD-5 and CKD-4 patients had higher TSAT, CRP, Hepcidin and markedly raised serum ferritin levels (Table 1).

The hepcidin level, Ferritin level, CRP level, Transferrin Saturation levels were observed to be higher in CKD-5 group with Iron than CKD-5, CKD-4 without Iron . Hepcidin levels correlated

significantly with Ferritin levels [rho=0.589, p< 0.0001). Hepcidin levels correlated with Serum CRP [rho = 0.176] with no significant difference p>0.05). Ferritin levles correlated significantly with CRP levels [rho = 0.510, p< 0.0001) (Table 2) (Figure 1, Figure 2 & Figure 3).

Table 1: Characterization of various parameters in CKD patients and control subjects.

Disease	N	Hemoglobin	Transferrin Saturation	Ferritin	CRP	Mean Hepicidin	Mean MAMC	Mean Albumin	Mean TIBC	Mean Iron
CKD-4 without Iron	10	8.7900±1.65089	27.1080± 14.00924	215.00± 40.969	18.34± 16.334	27.6900± 13.04611				
CKD-5 without Iron	22	8.4318±1.92388	20.7186± 8.33124	275.36± 42.639	20.98± 16.059	66.6727± 40.91265				
							19.33 ± 2.99.61 (cm)	3.24± 0.50g/dl	263.91 ± 116.8	66.18 ± 27.4 g/dl
CKD-5 with Iron	18	8.2778±1.90466	35.6783±11.80983	401.94± 68.209	31.82± 30.878	116.3444± 65.86968				
Control	5	14.5400±1.16103	31.7400± 6.41779	43.20± 8.468	0.96±0.329	71.1691± 58.09552				

Table 2: Correlations between CRP, Hepcidin and Ferritin levels

Correlations				
		Serum CRP	Serum Ferritin	Serum Hepcidin
Serum CRP	Pearson Correlation	1	.510**	.176
	Sig. (2-tailed)		.000	.197
	N	55	55	55
Serum Ferritin	Pearson Correlation	.510**	1	.589**
	Sig. (2-tailed)	.000		.000
	N	55	55	55
Serum Hepcidin	Pearson Correlation	.176	.589**	1
	Sig. (2-tailed)	.197	.000	
	N	55	55	55

**. Correlation is significant at the 0.01 level (2-tailed).

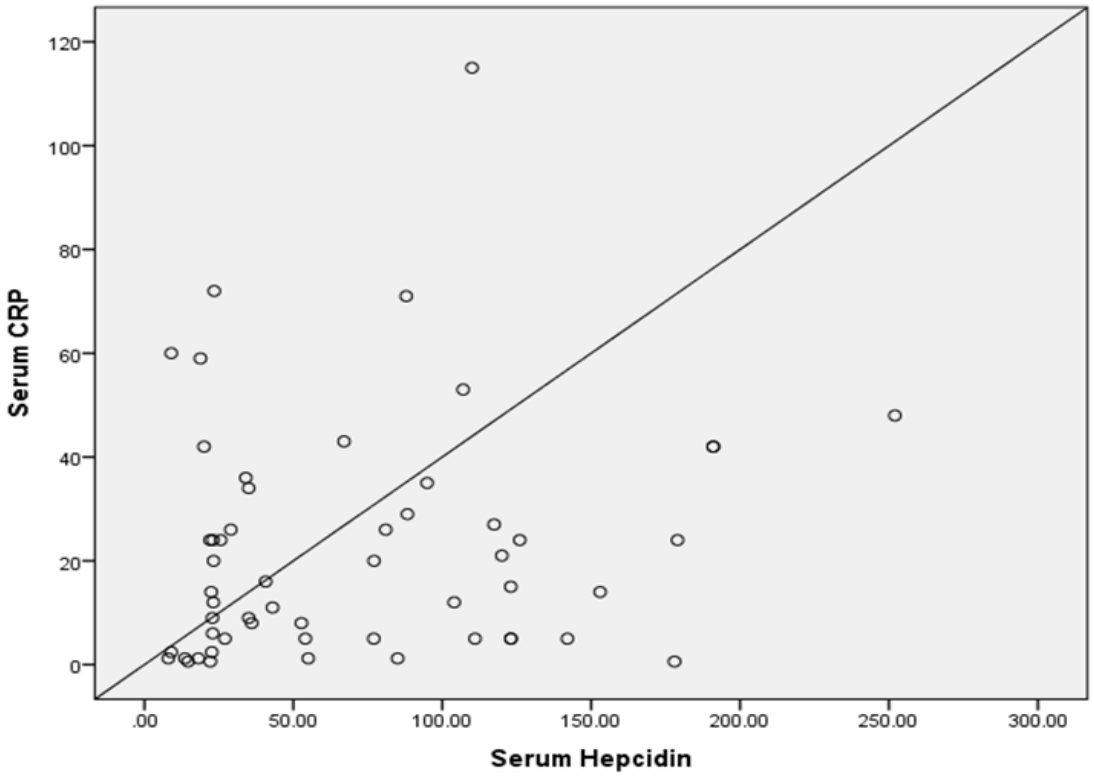

Fig. 1: Correlation between serum hepcidin & serum CRP [Scatter diagram]

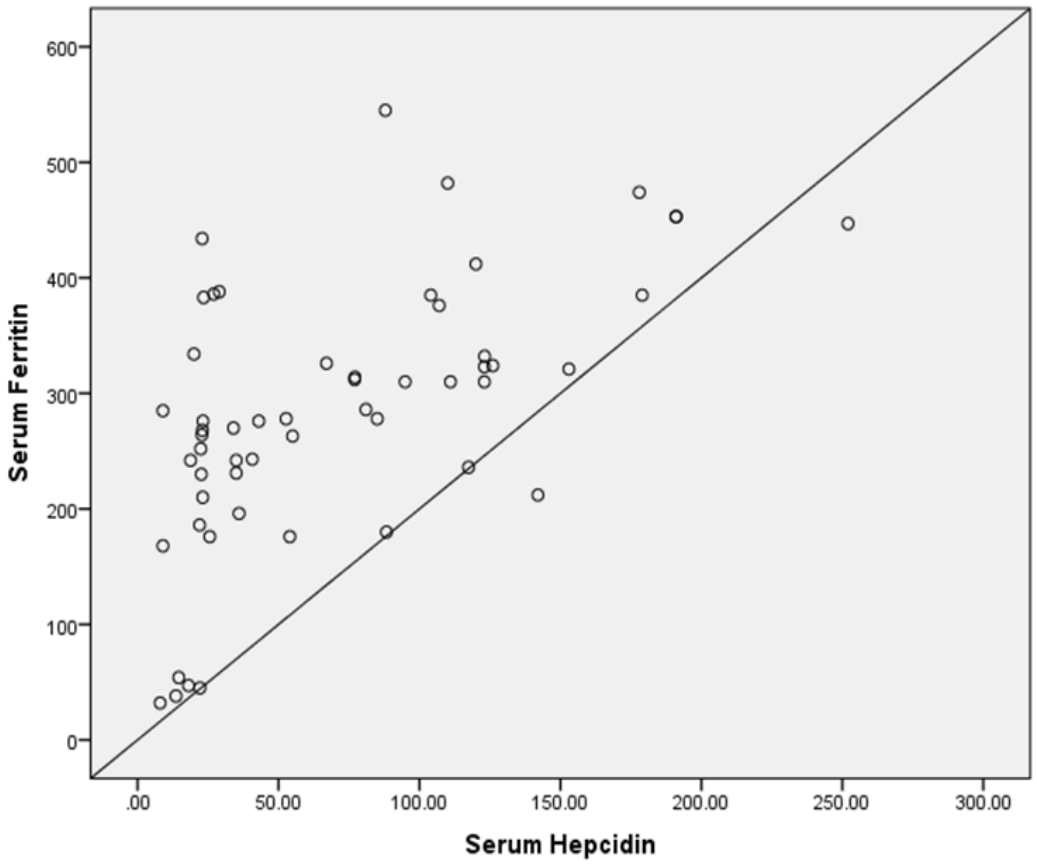

Fig. 2: Correlation between serum hepcidin and serum Ferritin [Scatter diagram]

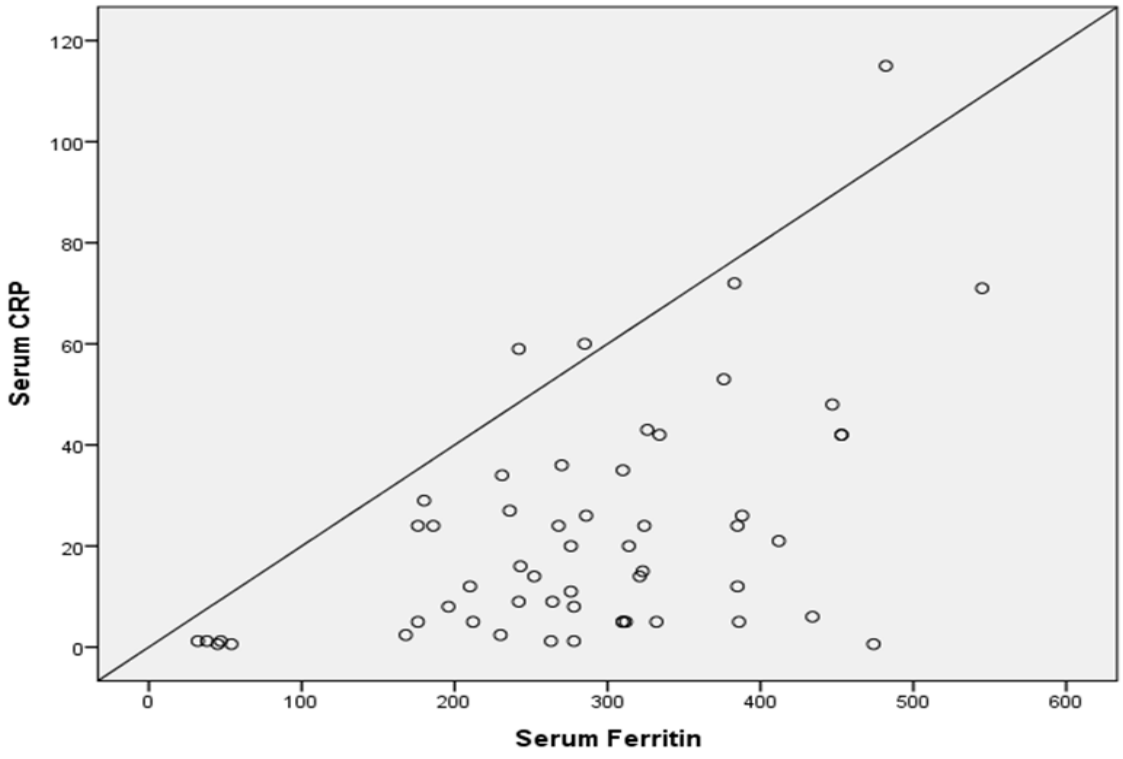

Fig. 3: Correlation between serum Ferritin & serum CRP [Scatter diagram]

Discussion

Iron deficiency is an important contributor to morbidity and mortality in ESRD. The cause is thought to be due to poor nutrition stemming from a predominantly vegetarian diet. Hepcidin, a regulator of body iron stores, has been postulated to play a critical role in the pathogenesis of anemia of chronic disease. Indian subjects have demonstrated to have micro inflammation and higher body fat percentage in healthy subjects as compared to Caucasians [8,9].

Hemoglobin levels in CKD-4/5 with/with out Iron intake shows less when compare with control subjects. Transferrin Saturation levels in CKD-5 with Iron intake shows high when compare with CKD-4 without Iron, CKD-5 without Iron and control subjects. Ferritin levels in CKD-5 with Iron intake shows very high when compare with CKD-4 without Iron, CKD-5 without Iron and control subjects. CRP levels in CKD-5 with Iron intake shows very high when compare with CKD-4 without Iron, CKD-5 without Iron and control subjects. Hepicidin levels in CKD-5 with Iron intake shows very high when compare with CKD-4 without Iron, CKD-5 without Iron and control subjects.

In our study there has been inflammatory activation with S.CRP levels increased in all study group. The cause of elevated mean CRP in subjects who did receive parenteral iron are on Hemodialysis is probably due to exposure to dialysis membranes, activation of immune cells (monocytes & T cells), or co-existing subclinical infection. A similar study done by V. Jha et al revealed inflammatory activation which was evident in 74 ESRD individuals has shown significantly higher CRP with (p value of < 0.001)[10]. Similar findings were noted in our study with significant correlation with hepcidin and serum CRP with significant p value (< 0.00).

In this study, the major finding was elevated hepcidin, in the study groups with subjects who did receive parenteral iron showed a higher S. hepcidin compared with subjects who didn't receive parenteral iron and control with significant (p value of < 0.01). A study done by Jha et al, revealed higher S. hepcidin levels in 74 ESRD patients with statistically significant (p value of < 0.12)[10]. Similar findings were noted in our study with increased serum hepcidin levels in ESRD individuals. Malyszko et al, revealed increased hepcidin level following I.V. iron therapy in Hemodialysis patients, but did not measure other inflammatory marker [11]. Comparing this study with our study group mean values of serum hepcidin was increased in CKD patients who were on haemodialysis and received i.v. iron therapy which was significant.

In our study S. ferritin was significantly elevated in subjects who did receive parenteral iron compared to individuals who didn't receive parenteral iron with statistically significant (p value of < 0.000). Ferritin was markedly increased due to iron overload in these subjects who did receive parenteral iron. V. Jha et al revealed elevated s. ferritin in individuals who did receive i.v. iron compared to individuals who didn't receive i.v. iron with p value (< 0.007)[10]. These findings were consistent with our study group individuals which showed significant statistically correlation between hepcidin and serum ferritin.

In our study there was no significant correlation between hemoglobin levels and s hepcidin levels. (p=0.138). Our study population consisted of only stage 4 and 5 CKD, the mean Hb levels were similarly low in all the groups of patients and had no relation to increasing s hepcidin levels. In our study, there was no significant correlation between hepcidin levels and TIBC (p=0.523). Serum Hepcidin & MAMC levels doesn't showed significant correlation (p=.240) could be established between these variables. Hepcidin & albumin levels did not show any correlation (p=0.511).

However in our study though CRP was significantly raised in the hemodialysis group, it is not correspondingly correlate with the elevated s hepcidin levels we had noted in this group.

Determination of hepcidin levels in CKD patients may not provide more diagnostic value than ferritin, but further studies are needed. Hepcidin and its regulatory pathways are potential therapeutic targets, which could lead to effective treatment of anemia of chronic disease and ESA hyporesponsiveness in CKD.

Atherosclerosis could induce an increase of the arterial IMT and arterial stiffening, and eventually lead to luminal obstruction with consequent ischemic events, such as myocardial infarction and stroke. Thus higher s hepcidin levels may be able to predict the subgroups of population with CKD who may be at higher risk for cardiovascular disease. This is an exciting area that needs to be further studied and may point the direction in which future strategies employing hepcidin as a diagnostic modality or as a target of directed therapeutic approaches.

Conclusion

There have been elevated serum hepcidin levels in ESRD patients more in those receiving Iron therapy.

High hepcidin levels would reveal functional iron deficiency. There was significant correlation between levels of hepcidin and iron status with inflammatory markers. The cause of the relatively greater degree of inflammatory activation as well as the relationship with parenteral IV iron administration needs further studies. Larger randomized multicenter studies could throw more light on the diagnostic and therapeutic potentials of using Hepcidin-25 levels in regular practice.

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Author Affiliation:
*Department of Urology, **Department of Obstetrics & Gynaecology, *** Department of Surgery, Bharti Hospital and Research Center, Pune-Satara Road, Dhankawadi, Pune-411043, Maharashtra, India.

Reprint Request:
Sanjay Prakash Dhangar, B-704, Aster Trinity, Sai baba nagar, Survey no 62, KondhwaKhurd, Pune – 411048, Maharashtra, India.
E-mail: sanjayamrapali18@gmail.com

Surgical Therapy in Infertile Men with Obstructive Azoospermia Due to Ejaculatory Duct Obstruction–Outcome after Transurethral Resection of Ejaculatory Duct

Sanjay Prakash Dhangar*, Ibrahim H. Kothawala*, A.D. Gosavi, Arefakothawala**, Abhay Kumar***, Sachin Patil***

Abstract

Objectives: To report our experience with transurethral resection of ejaculatory ducts (TURED) in infertile men with symptomatic ejaculatory duct obstruction. *Material and Method:* We studied 4 cases of ejaculatory duct obstruction from January 2014 to January 2015. Investigations included a history, physical examination, semen analysis, semen culture, hormone levels and trans-rectal ultrasonography. Results were evaluated after TURED, especially the semen parameters, the patency and the pregnancy rate. *Results:* Causes of obstruction were previous infection with inflammation, calculus and prostatic cyst. Mean age was 34 years.Mean Duration of infertility was 3.5 years.Mean FSH value was 1.5. Mean Semen volume was 0.5 ml with SD of ± 0.10m. In all patients fructose was absent on semen analysis. On TRUS mean seminal vesicle diameter was 16.00 mm Seminal vesicle was dilated in all cases. In 3/4 patients who had patency in follow up had a mean sperm count of 14.33 million/ml.Mean time to patency was 3 months.Post TURED ejaculate volume improved in all patients with mean of 1.75ml and SD of 0.50 ml. Patients who had undergone TURED reported a patency rate of 75 %, with one patient showing absence of sperms in follow up. After TURED on follow up pregnancy was reported in one case i.e. 25 %. In our study mean follow up was 10 months. *Conclusions:* Men with symptomatic EDO who underwentTURED showed improvements in theirejaculation, sensation of orgasm, semenanalysis values and fertility.

Keywords: Ejaculatory Duct Obstruction; Infertility; Ejaculatory Duct/Prostatic Cyst; Transurethralresection of Ejaculatory Duct; Tured.

Introduction: Infertility by itself does not threaten the life, but it has devastating psycho-social consequences on infertile couples. It remains a worldwide problem and challenge. Management of infertility has been and still a difficult medical task not only because of the difficulty in the diagnosis and treatment of the reproductive disorders in each partner, or the poorly unstated interaction between the partners' fertility potentials, but also because of the fact that success of treatment is clearly identifiable entity - the achievement of pregnancy. By following the *evidence-based* management protocol infertile couples will have a good chance to start up their treatment in the proper way at early time [1] .

Azoospermia, defined as complete absence of sperm from the ejaculate, is present in less than 2% of all men and in 15% of infertile men [2].

Among infertile men, the incidence of azoospermia is about 10 to 15%, of which 40% is due to obstructive azoospermia [3].

Causes of obstructive azoospermia are vasectomy, congenital absence of vas deferens and ejaculatory duct obstruction, and acquired diseases (eg,epididymal obstruction secondary to infection, vasal injury due to previous inguino-scrotal surgery) [4].

There are few diagnoses in azoospermic men that

are amenable to surgical correction. The most common among these are epididymal obstruction, vasectomy and obstruction of the ejaculatory ducts. The importance of diagnosing these conditions and treating them appropriately lies in the fact that they can be cured [4].

Ejaculatory duct obstruction (EDO)is reported to be the cause of azoospermia in up to 5% of patients [5].

In the present study we assessed and providedthe evidence for the effectiveness of surgical treatment of ejaculatory duct obstruction.

Objectives

To report our experience with transurethral resection of ejaculatory ducts (TURED) in infertile men with symptomatic ejaculatory duct obstruction and to assess the patency rate after trans-urethral resection of ejaculatory ducts.

Material and Methods

This study was carried out in Bharti Hospital and Research Center, Pune during the periodJanuary 2014 to January 2015. This study was done on 4 patients of Obstructive Azoospermia who were subjected to Transurethral resection of Ejaculatory ducts (TURED).

Ethics

The study protocol was reviewed and approved by institutional ethical and scientific committee and informed consent was obtained.

Statistics

Data analysis was carried out under the guidance of our statistics expert, using Statistical Package for Social Sciences version 17.

The Inclusion Criteria for TURED

1. The preoperative symptoms likeinfertility, non-projectile ejaculation, a decrease in sensation of orgasm, and/or pain with ejaculation, history of prostatitis or epididymitis, perineal or testicular pain.
2. Low volume ejaculate
3. Bilateral palpable vas deferens
4. Normal hormonal profile
5. Dilatedseminal vesicles on trans-rectal ultrasonography (TRUS)

The preoperative symptoms included infertility, non-projectile ejaculation, a decrease in sensation of orgasm, and/or pain with ejaculation etc. Investigations included a focused history and physical examination, two semen analyses, a semen culture (with PCR analysis), and TRUS. On TRUS, each man was evaluated for prostatic calcifications, ejaculatory duct cysts and the diameter of the seminal vesicles. Seminal vesicles were considered dilated when they were more than 15mm in diameter.

Surgical Technique

All operations were done under regional anaesthesia. Cystoscopy was performed and the bladder was inspected. Cysto-urethroscopy findings such as midline cysts and altered verumontanum anatomy etc. were recorded. A drape is used with a finger in the rectum to allow better depth perception and visualization of the posterior prostate. Using a transurethral resection set, cystic lesions were opened. If an ejaculatory duct cyst is present, it is usually deep and just posterior to the verumontanum. Therefore, the verumontanum is deeply resected with care not to injure the rectum. Once efflux from the ejaculatory ducts of copious cloudy material is present, the resection was considered adequate. Electrocautery is used judiciously to avoid occlusion of the newly opened ejaculatory ducts. Care is taken at all times to protect the bladder neck and external sphincter from injury that might result in retrograde ejaculation and urinary incontinence. A Foley catheter is left overnight and the patient was discharged next day. All the patients received a 5- to 7-day course of antibiotics. Vasogram or seminal vesiculogram were not done.

Postoperative Evaluation

All patients were assessed at 6 weeks and 3 months and included focused history about improvements in symptoms of EDO andsemen analyses and continued until pregnancy was achieved. Patency was defined as the presence of motile sperm in the ejaculate of at least one postoperative semen sample. Pregnancy was defined as unassisted establishment (no assisted reproduction) of a viable pregnancy leading to a live birth. Follow-up information was obtained from clinic visits and telephone contact.

Results

Etiology

In 50 % of patients with ejaculatory duct obstruction had history of previous infection with inflammation as a cause of obstruction.Other 25% had calculus and prostatic cyst as obstructive etiology [Figure 1].

Descriptive Statistics (Pre-operative parameters) [Table 1]

In 4 Patients who has Undergone TURED

i. Mean age was 34 with standard deviation of ± 2.65 with minimum age of patient seen was 32 and maximum age was 38 years.

ii. Mean Duration of infertility was 3.5 years with standard deviation of ± 2.38. Minimum duration of infertility was 1 year and maximum was 6 years.

iii. Mean FSH value was 1.5 with SD of ± 0.58

iv. Mean Semen volume was 0.5 ml with SD of ± 0.10ml. Minimum semen volume was 0.4 ml and maximum was 0.6 ml.

Fructose in Semen Analysis

In all patients who underwent TURED due to ejaculatory duct obstruction fructose was absent on semen analysis.

Patency in TURED Patients

Patients who had undergone TURED reported a patency rate of 75 %, with one patient showing absence of sperms in follow up.

Pregnancy Rate Post TURED

After TURED on follow up pregnancy was reported in one case i.e. 25 %.

Descriptive Statistics (Post-Operative Parameters) [Table 2]

In 4 Patients who has Undergone TURED

i. Mean operative time was 77 minutes with SD of ± 9.57. Minimum operative time required was 70 minutes and maximum operative time was 90 minutes.

ii. In 3/4 patients who had patency in follow up had a mean sperm count of 14.33 million/ml with SD of ± 6.81.Minimum sperm density seen was 9 million/ml and maximum was 22 million /ml.

iii. Mean time to patency was 3 months with SD of ± 1.0 .Minimum time required for patency was 2 months and maximum time was 4 months.

iv. In our study mean follow up was 10 months with SD of ± 1.63 .Minimum follow up was 8 months and maximum was 12 months.

v. On TRUS mean seminal vesicle diameter was 16.00 mm with SD of 0.82 mm.One patient had diameter of 17 mm while others have diameter more than 15 mm. Seminal vesicle was dilated in all cases.

vi. Post TURED ejaculate volume improved in all patients with mean of 1.75ml and SD of 0.50 ml.

Complications with TURED are rare if the procedure is done carefully and with expertise. In our study, overall there were no complications associated with the procedure.

Table 1: Descriptive Statistics (Pre-operative parameters)

	N	Minimum	Maximum	Mean	Std. Deviation
Age	4	32	38	34.50	2.65
Duration of Primary infertility(years)	4	1yr	6 yrs	3.50	2.38
FSH	4	1.0	2.0	1.50	0.58
Semen volume	4	0.4ml	0.6ml	0.53	0.10

Table 2: Descriptive Statistics (post-operative parameters)

	N	Minimum	Maximum	Mean	Std. Deviation
Operative time	4	70	90	77.50	9.57
Sperm density (million/ml)	3	9	22	14.33	6.81
Time to patency (month)	3	2	4	3	1
Follow up(month)	4	8	12	10.00	1.63
On TRUS Seminal Vesicle in Millimeters (mm)	4	15	17	16.00	0.82
Post TURED ejaculate vol.	4	1	2	1.75	0.50

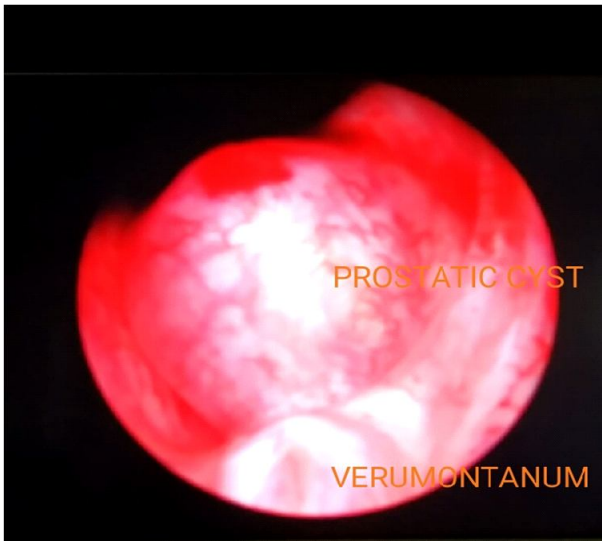

Fig. 1:

Discussion

For patients with obstructive azoospermia, surgical therapy is an acceptable management option in comparison with assisted reproduction techniques (ART) such as ICSI, which tend to bypass the male factor etiology. Surgical correction offers a long-term solution and aims at correcting the underlying pathology. It also obviates the need for repeated ART each time the individual wishes to contribute to a pregnancy.

Ejaculatory duct obstruction is a rare cause of infertility, but it is essential to diagnose it, as it can be easily corrected with a minor cystoscopic procedure. Detecting Ejaculatory duct obstruction has become easier and less invasive with the development of high resolution TRUS, which by itself has been shown to be very effective for identifying possible EDO. It can show cysts or calcifications that might cause blockage, and identifies dilated seminal vesicles.

In patients with suspected EDO, TURED has become the standard procedure. It was described by Farley and Barnes in 1973 [6].

Etiology

Etiology of EDO is varied. Ejaculatory duct obstruction can be either congenital or acquired. Congenital causes include congenital atresia or stenosis of the ejaculatory ducts and utricular, mullerian, and wolffian duct cysts. Acquired causes may be secondary to trauma, either iatrogenic or otherwise, or infectious or inflammatory aetiologies. Calculus formation secondary to infection may also cause obstruction [7]. In our study 50 % of patients with ejaculatory duct obstruction had history of previous infection with inflammation as a cause of obstruction.Other 25% had calculus and prostatic cyst each as obstructive etiology.Paick J et al [8] in his study of 50 men the main cause of EDO was a midline cyst in 16, Wolffian malformation in four, tuberculosis in 17, previous genitourinary infection in five and idiopathic in eight men. In 17 patients the seminal vesicles appeared to be atrophied on TRUS; 15 of these patients had a history of pulmonary tuberculosis and subsequent vasography in five showed multiple bilateral vasal obstruction. Tu XA et al [9] in their study had 6 prostatic cyst and 3 cases of calcification out of total 60 cases.

Age

Patients of EDO are usually young individuals. In our study mean age was 34 with standard deviation of ± 2.65 with minimum age of patient seen was32 and maximum age was 38 years.Ozgok Y et al [10] in his study of 24 patients had mean age of 29 years with variation between 20 and 40 years (mean=29).Kochakarn W et al [11]in his study of 7 patients has age range from 32-45 years old (mean 34.5).

Duration of Infertility

In our study duration of infertility was 3.5 years with standard deviation of ± 2.38.Minimum duration of infertility was 1 year and maximum was 6 years.

Serum FSH Levels

Mean FSH value was 1.5 with SD of ± 0.58 in our study. Kochakarn W et al [11] in his study of 7 patients had seventy-one per cent patients with normal hormonal profiles and twenty-nine per cent had a slight increase of FSH, LH but not more than one fold of normal range.

Semen Values

Mean Semen volume was 0.5 ml with SD of ± 0.10ml in our study. Minimum semen volume was 0.4 ml and maximum was 0.6 ml. in the study by Christopher W et al R [12], the mean ejaculate volume before TURED was 1.1 mL. In study by Immo-Schroeder-Printzen et al [13], sperm analysis pre-operatively demonstrated typical low-volume ejaculates with azoospermia, ejaculate volume was between 0.5 and 1.6 ml (mean 0.95 ml) and seminal

pH was acidic in every case.

Fructose in Semen Analysis

In all patients who had undergone TURED due to ejaculatory duct obstruction fructose was absent on semen analysis.Immo-Schroeder-Printzen et al [13] in their of 16 patients with EDO,fructose was absent or below 13 μmol/ejaculate. Testicular volumes, serum FSH and spermatogenesis (Johnson score >8) were normal in all cases.

TRUS Findings

TRUS is certainly the easiest way to detect cystic lesions at the verumontanum level as well as dilatations of the internal ductal diameter and of the seminal vesicles. On TRUS mean seminal vesicle diameter was 16.00 mm with SD of ±0.82 mm in our study.Christopher W et al [12] On TRUS, each man was evaluated for prostatic calcifications, ejaculatory duct cysts and the diameter of the seminal vesicles. Seminal vesicles were considered dilated when they were = 12 mm in diameter. In study by Immo-Schroeder-Printzen et al [13] TRUS findings cover midline utricular and Müllerian cysts , as well as dilatated ejaculatory ducts, defined with an internal ductal diameter >2.3 mm and / or dilatated seminal vesicles with a cross-sectional diameter >15 mm.

Operative Time

Mean operative time was 77 minutes with SD of ± 9.57. Minimum operative time required was 70 minutes and maximum operative time was 90 minutes.

Post Turedejaculate Volume

Post TURED ejaculate volume improved in all patients with mean of 1.75ml and SD of ± 0.50 ml. Christopher W et al [12]The mean ejaculate volume after TURED increased to 2.3 mL.

Sperm Count

In 3 / 4 patients who had patency in follow up had a mean sperm count of 14.33 million / ml with SD of ± 6.81.Minimum sperm density seen was 9 million / ml and maximum was 22 million / ml. In study by Christopher W et al [12], the total motile sperm count increased 38.1 million per ejaculate. Ozgok Y et al [10] in his study of 24 patients before transurethral resection mean sperm count was 1.66x10(6) / ml compared to 25.4x10(6)/ml postoperatively. Kochakarn W et al [11] in his study of 7 patients 6 of 7 (86%) showed improvement of semen analysis. Up to one year, 6 of 7 (86%) have normal semen analysis and another one still had azoospermia.

Time to Patency

Mean time to patency was 3 months with SD of ± 1.0. Minimum time required for patency was 2 months and maximum time was 4 months.

Patency in Tured Patients

Patients who had undergone TURED reported a patency rate of 75%, with one patient showing absence of sperms. Yurdakul t et al [14] in his study before TURED, all patients were azoospermic, following the operation, sperms were seen in the ejaculates of 11 / 12 patients. Immo-Schroeder-Printzen et al in their of 16 patients with EDO had post-operative ejaculates showing patency in all patients with Mullerian cysts. There was an improvement in sperm counts in all Mullerian cyst patients with successful opening but only an improvement in two patients without cystic lesions. The worst results were obtained in all patients with lateral cystic lesions of the ductus ejaculatorius.

Pregnancy Rate Post Tured

After TURED on follow up pregnancy rate was reported to be 25% with follow up period of 8 – 12 months. In study by Christopher W et al [12] four of the six men available for long-term follow-up reported successful paternity without assisted reproduction techniques. In study by Yurdakul t et al [14] after a mean follow-up period of 12 (range 4-36) months, five (41.6%) pregnancies were noted.Ozgok Y et al [10] in his study of 24 patients had a mean follow-up period of 9 (6-18) months, 6 (25%) pregnancies were noted. Kochakarn W et al [11] in his study of 7 patients had a long-term follow-up, 4 of 7 (57%) were able to impregnate their wives. TuXa et al [15] in his evaluation of43 men with EDO treated by TURED, 36 (83.7%) showed improved semen parameters and 11 (25.6%) achieved pregnancies. Weintraubet al [16] reported 25% pregnancy rate in their study of eight patients with ejaculatory duct obstruction.

Follow up

In our study mean follow up was 10 months with SD of ± 1.63. Minimum follow up was 8 months and maximum was 12 months.

Conclusion

Abnormalities of the distal ejaculatory ducts especially related to infertility have been well documented with the advent and increased use of high-resolution TRUS. In an infertile male with oligospermia or azoospermia with low ejaculate volume, normal secondary sex characteristics, testes, and hormonal profile, and dilated seminal vesicles, midline cyst, or calcification on TRUS, ejaculatory duct obstruction is suggested.EDO is a very treatable disease that can be cured with a simple cystoscopic procedure. This treatment has a positive impact not only on fertility, but also on sexual satisfaction. Men with symptomatic EDO who underwent TURED showed improvements in their ejaculation, sensation of orgasm, semen analysis values and fertility.

Genetic testing and counseling should be considered in appropriate cases, because genetic factors may impact both the patient and his potential offspring, also not all patients post successful TURED are able to impregnate their spouse.

Current technology often allows for paternity for men previously labeled sterile. In general, if possible, it is preferable to improve the male's fertility potential and allow the couple to conceive by intercourse. This is not only economic but also provides the males great mental satisfaction.

Limitations

This is a non-randomised clinical study to evaluate TURED results. The availability of necessary instruments and expertise made the study feasible. However, long term prospective trials are necessary to validate the durability of this therapy and its effect on the symptoms of EDO.

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Original Article

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Histological Pattern of IgA Nephropathy (IgAN) by Oxford Classification by MEST Scoring

Lakshminarayana G.R.*, Ranjit Narayanan, Raghunath K.V.*, Indu S.*, Seethalekshmy N.V.***, Biju M.V.******

Author Affiliation: *Department of Nephrology, EMS Memorial Cooperative Hospital and Research Centre, Perinthalmanna, Malappuram, Kerala, India. ******Department of Nephrology, KMCT Medical College, Manassery PO, Mukkam, Kozhikode, Kerala, India. *******Department of Pathology, Amrita Institute of Medical Sciences and Research Centre, Kochi, Kerala, India. ********Department of Nephrology, DM Waynad Institute of Medical Sciences, Meppadi, Waynad, Kerala, India.

Reprint Request:
Lakshminarayana G.R., Consultant Nephrologist, Department of Nephrology, EMS Memorial Cooperative Hospital and Research Centre, Perinthalmanna, Malappuram, Kerala, India-679322.
E-mail: drlng23@gmail.com

Abstract

IgAN is one of the commonest biopsy proven primary glomerular diseases with diverse histological patterns based on the geographic location. This is a study was done by including all consecutive cases of biopsy proven IgAN(native kidney) performed at EMS Memorial Cooperative Hospital, Perinthalmanna, Kerala, India, from September 2009 to February 2016. We had 62 (Females: 36, Males: 26) cases of biopsy proven IgAN. The mean age of the patients was 37.71 years and male: female ratio was 1.38:1. The IgAN was classified according to the Oxford classification (MEST scoring). Majority of the patients had mesangial hypercellularity (91.94%) and tubular atrophy (69.36%; T1-43.55%, T2-25.81%). Only few patients had endocapillary proliferation (20.97 %), and segmental sclerosis (39.68 %). Glomerular crescents (involving 5-20% of glomeruli) were found in 4.84 % of patients with IgAN. The commonest combinations of MEST scoring were M1, E0, S0, T1 (25.81%), followed by M, E0, S1, T1 (14.52%) and M0, E0, S0, T0, was seen only in 6.45% of cases. This study confirms the aggressive nature of IgAN in Indian subcontinent, unlike western literature.

Keywords: IgAN; Oxford-MEST Classification.

Introduction

Immunoglobulin A nephropathy (IgAN) is one the most common glomerulonephritis worldwide [1-6]. The bulk of the disease burden is borne by Asian countries [2-6]. The Oxford classification (MEST) of IgAN was proposed in 2009; found that mesangialcellularity, endocapillary proliferation, segmental sclerosis and tubular atrophy / interstitial fibrosis, to have independent predictive value on clinical outcome [7] Recent trials from Europe and North America have validated its utility [8-10]. However, its clinicopathologic spectrum in Asian and South American countries is not well documented except for few studies [11-14].

Aims and Objectives

To classify the patients of biopsy proven IgAN based on MEST Oxford- classification.

To analyze the histological combinations of MEST scoring in IgAN.

Meterials and Methods

This is a retrospective study which included all consecutive patients with diagnosis of IgAN on light and immunofluorescence microscopy. The biopsies performed at EMS Memorial Cooperative Hospital and Research Centre, Perinthalmanna, Kerala, from September 2009 to April 2016, under guidance of ultrasound using Bard® Max-Core® disposable core biopsy instrument, CR Bard Inc., USA. All the biopsies were analyzed by light microscopy using hematoxylin and eosin (H&E), periodic acid Schiff (PAS), Jone's silver methaneamine and Gomori's trichrome stains (MT) and immunofluorescence studies were performed using anti-human IgG, IgA, IgM, C3, C1q, kappa and lambda light chains. The IgAN was diagnosed in presence of IgA-dominant mesangial or immune deposits (> 2+ and the absence of C1q deposition) through immune fluorescence (IF) microscopy. The data was analyzed by SPSS 17 for Windows, by SPSS Inc. IL, USA. Two-sided p value of < 0.05 was considered as statistically significant.

The IgAN was classified according to the Oxford-MEST classification [mesangial hypercellularity score (M; M0 < 0.5, M1 >0.5), the presence of endocapillary proliferation (E; E0: absent, E1: present) and segmental glomerulosclerosis / adhesion (S; S0: absent, S1: present), and the severity of tubular atrophy / interstitial fibrosis (T; T0 <25%, T1: 26–50%, T2 >50%) [7].

Results

A total of 271 patients underwent renal biopsy during the study period. The IgAN was diagnosed in 62 out of 271 (22.88%) patients, and was most common biopsy proven renal disease in the study. Among those with IgAN, 36 were males and 26 were females; with M: F ratio of 1.38:1. The age of subjects ranged from 12-75 years (Mean:37.71, SD: 14.21) and majority (62.90%) were of < 40 years of age. Both males and females were of similar age (Table 1); the difference was statistically insignificant (p:0.20).

The indications of renal biopsies in the study were; microhematuria in 3, subnephrotic protienuria in 3, subnephrotic proteinuria with haematuria in 13, nephrotic syndrome in 4 and renal insufficiency (serum creatinine >1.4mg / dl) with proteinuria and haematuria in 39 subjects. Among those with renal insufficiency 28.21 % (11 out of 39) had severe failure (eGFR < 30 ml / min / 1.73m2), 69.23 % (27 out of 39) had moderate renal insufficiency (eGFR 30 to 59 ml / min / 1.73m2) and 2.56 % (1 out of 39) had mid renal insufficiency.

The patients were categorised based on indication for renal biopsies and results of MEST scoring are represented in figures 1 to 6. Frequencies of MEST score combinations in the study are listed in Table 2. Overall prevalence of M1, E1, S1, T1 and T2 was noted in 91.94, 20.97, 39.68, 43.55 and 25.81% of renal biopsies respectively. The commonest combinations of MEST scoring were M1, E0, S0, T1 (25.81%), followed by M, E0, S1, T1 (14.52%) and M0, E0, S0, T0, was seen only in 6.45% of cases. 3 out of 62 (4.84 %) patients also had associated extracapillary proliferation in the form of fibrocellular crescents in renal biopsy. There was statistically significant correlation of indication of biopsy with presence of M1 (p:0.008) and TA (tubular atrophy T1 or T2) (p:0.018) on univariate analysis, however, it was insignificant with E1 (p:0.374) or S1 (p:0.295).

Multivariate analysis of indication of biopsy with MEST scoring showed a statistically significant correlation with presence of TA (p:0.003); whereas, it was not significant with M1 (0.689), E1 (0.272) or S1 (0.30). There was no statistically significant effect of gender or age on MEST score on multivariate analysis.

Mesangial Hypercellularity

The majority of the subjects had mesangial hypercellularity (M1) in the study.The majority of patients who underwent renal biopsy for microhematuria (66.67%), subnephrotic protienuria (100%), subnephrotic proteinuria with haematuria (92.31%), nephrotic syndrome (50%) and renal insufficiency with proteinuria and haematuria (97.44 %) had M1.

Endocapillary Proliferation

Only minority of patients had endocapillary proliferation (E1) in the study. The E1 was found in 38.46% of subjects with subnephrotic proteinuria with haematuria, 25% with nephrotic syndrome and 17.95% with renal insufficiency with proteinuria and haematuria. None of the subjects with isolated microhematuria orsubnephrotic protienuria had E1.

Segmental Glomerulosclerosis

The segmental glomerulosclerosis (S1) was found 39.68 % in the study. The S1 was found in 33.33% of subjects with isolated microhematuria, 66.67% with subnephrotic protienuria, 23.07 % with

subnephrotic proteinuria with haematuria, 75% with nephrotic syndrome and 48.72% with renal insufficiency with proteinuria and haematuria.

Tubular Atrophy

Tubular atrophy (TA) of grade either T1 or T2 was found in 69.36% of biopsies. The presence of TA was found in 33.33% of subjects with isolated microhematuria, 33.33% with subnephrotic protienuria, 56.16% with subnephrotic proteinuria with haematuria, 50% with nephrotic syndrome and 84.62% with renal insufficiency with proteinuria and haematuria.

Table 1: The demographic data of subjects with IgAN (age)

Gender	N	Mean	Std, Deviation	Std. Error
Females	26	35.96	13.55	2.66
Males	36	38.97	14.73	2.45
Total	62	37.71	14.21	0.95

Table 2: Frequencies of MEST score combinations

• M1 E0 S0 T1: 25.81 %	• M1 E1 S0 T0: 0.3 %
• M1 E0 S0 T0: 9.68 %	• M1 E1 S0 T1: 0.1 %
• M1 E0 S1 T1: 0.1 %	• M1 E1 S0 T2: 6.45 %
• M1 E0 S0 T2: 0.1 %	• M1 E1 S1 T0: 0.1 %
• M1 E0 S1 T0: 9.68 %	• M1 E1 S1 T1: 4.76 %
• M1 E0 S1 T1: 9.68 %	• M1 E1 S1 T2: 0.3 %
• M1 E0 S1 T2: 14.52 %	• M0 E0 S0 T0: 6.45 %

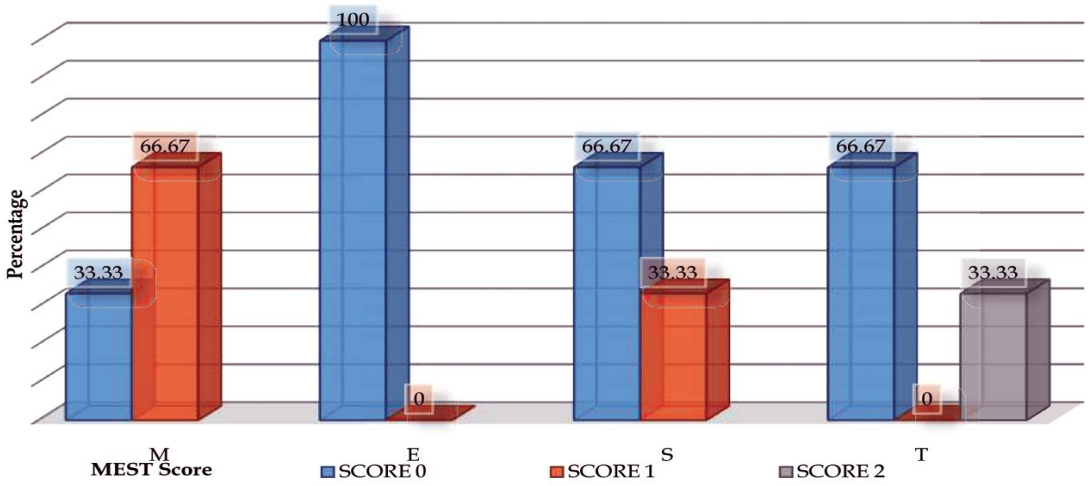


Fig. 1: Mest score in those microhematuria

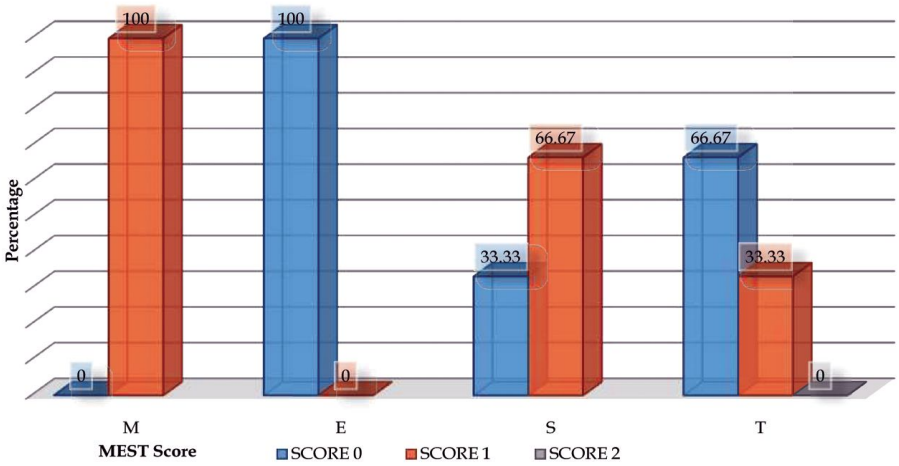


Fig. 2: MEST in those with proteinuria

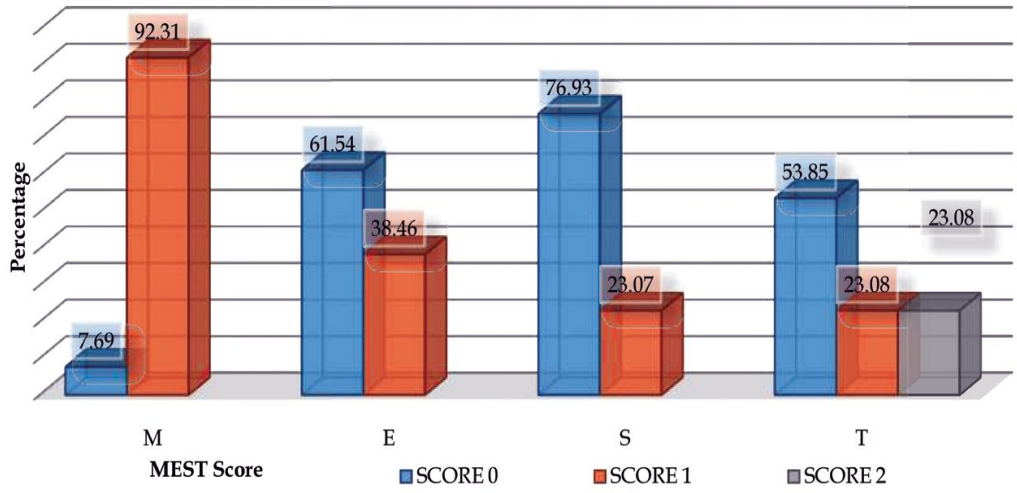


Fig. 3: MEST score in those with protienuria and microhematuria

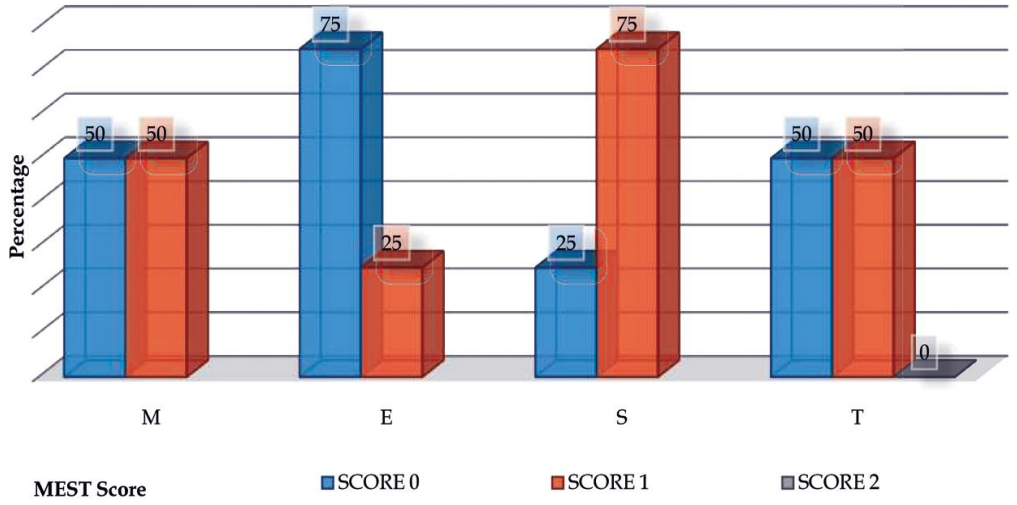


Fig. 4: Mest score in those with nephrotic syndrome

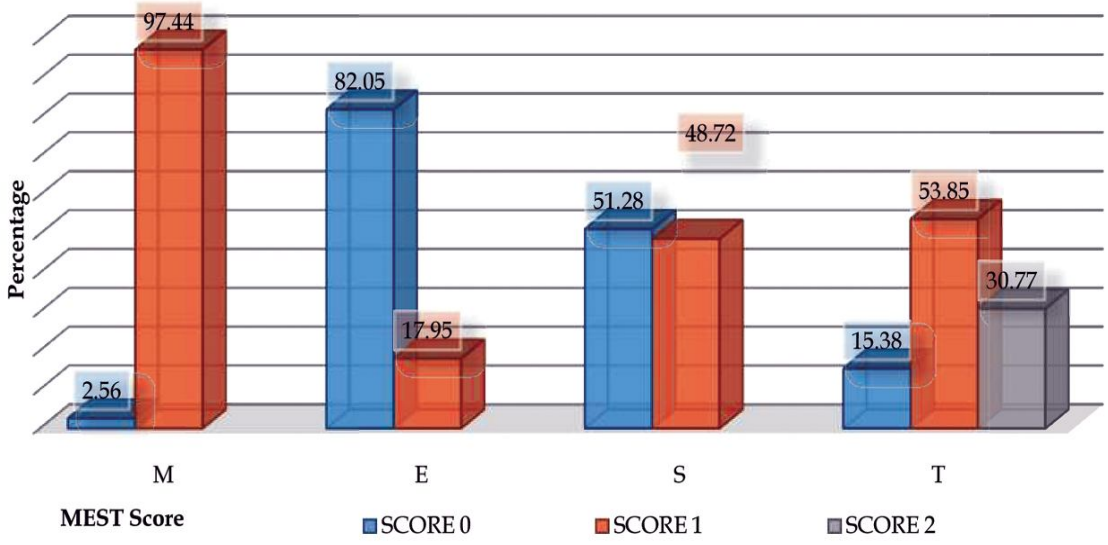
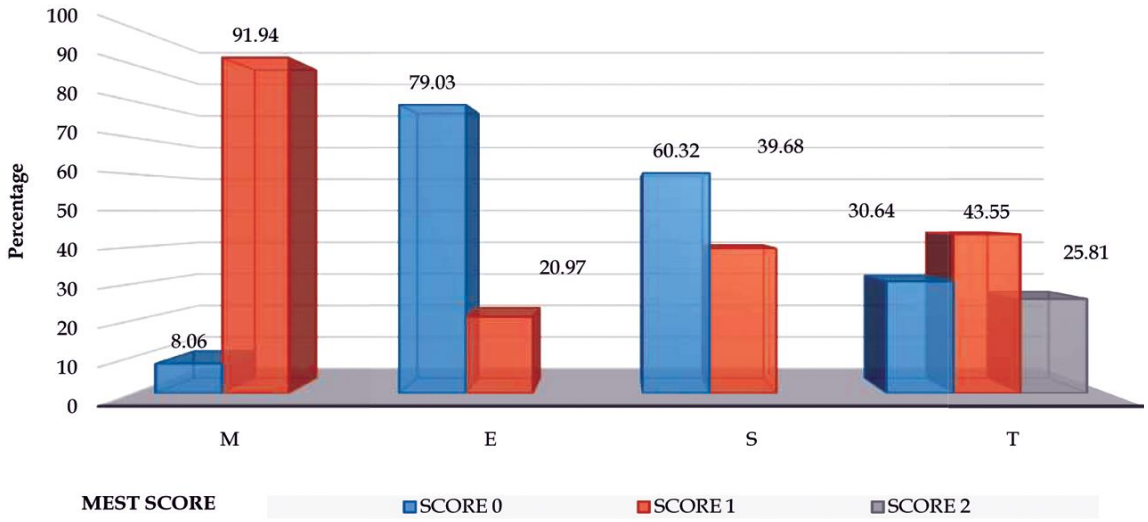


Fig. 5: Mest score in those with Renal Insufficiency



Fug. 6: Mest score of the all subjects study

Discussion

IgAN is among the most common glomerular diseases worldwide, with varying histological patterns [1-6]. Oxford classification of IgAN by MEST scoring, is an important step for classification as it will serve to achieve for uniformity in classification, initiation, monitoring of treatment [7]. It will also aid easier design of multicentre trials and analysis.

In our study the IgAN was the most common biopsy proven renal disease; more common in males than females and majority of the subjects were of age < 40 years. The presence of renal insufficiency with proteinuria and haematuria was the commonest indication for renal biopsy (62.90%) followed by subnephrotic proteinuria with haematuria (20.97%), nephrotic syndrome (6.45%),microhematuria (4.84%) and subnephrotic protienuria in 4.84 % of subjects. Overall prevalence of M1, E1, S1, T1 and T2 was noted in 91.94, 20.97, 39.68, 43.55 and 25.81% of renal biopsies respectively.

In of the earlier studies from India; involving 66 patients (male: female ratio of 4.4:1; mean age: 29.9 years), the prevalence of MEST scores M1, E1, S1, T1 and T2 were observed in 68.18%, 24.24%, 48.48%, 30.30% and 43.93% in respectively [11].

In a study from Iran (102 patients,72% males, mean age: 37.7 ± 13.6 years) the rates MEST variables were; M1: 90.2 %, E: 32 %, S: 67 %, T in grades 1 and 2 were in 30% and 19% respectively [12].

In a Brazilian study (600 patients; Male to female ratio: 1.24:1; mean age of 32.76 ± 15.12 years); M1 and S1 were the main glomerular findings (47.6 and 46.2 %). T1 or T2 was observed in 32.2% of the cases. Segmental sclerosis (S1) showed a stronger tendency of association with the presence of tubulointerstitial lesions (T1 and T2) than other glomerular variables.Tubular atrophy and interstitial fibrosis were more strongly associated with higher 24-h proteinuria and serum creatinine levels [13].

The findings of the present study are consistent with first two studies and patients in Brazilian had milder form of disease with mean serum creatinine level of 1.5 mg/dl with lesser of them having mesangial hypercellularity and tubular atrophy [11, 12, 13].

Most patients in the present study presented with renal failure similar to profile of earlier studies [11, 12] and except that a significant percentage (23%) also had nephrotic range proteinuria in one of them [11]. The present study being a hospital based one, might led to the bias in selection for biopsies towards those with renal insufficiency similar to other studies. [11, 12].

The commonest combinations of MEST scoring in the study were M1, E0, S0, T1 (25.81%), followed by M, E0, S1, T1 (14.52%) and M0, E0, S0, T0, was seen only in 6.45% of cases. Whereas; the commonest combination was M0 E0 S0 T0 (22.4%) in Brazilian study, expectedly as patients had milder form of disease [13].

In the present study, of the MEST variables presence of M1, TA (T1 or T2) had a statistically significant correlation with indication for biopsy on univariate analysis, however; only on TA showed a statistically significant correlation on multivariate analysis. There was no statistically significant effect of gender or age on indication for renal biopsy or MEST score on multivariate analysis.

In one of the earlier study; gender had a significant effect (males > females) was on presence of segmental glomerulosclerosis and interstitial fibrosis/ tubularatrophy. The possible reason for this difference being a higher serum creatinine and protienuria in males in that study [12].

Conclusions

IgAN is more common in males and affects those of younger age.Majority of patients who underwent biopsy had renal insufficiency; majority of those presenting with renal insufficiency had M1 and T1 or T2. Among the MEST score combinations M1 E0 S0 T1, was the commonestand M0 E0 S0 T0 was one the least common patterns, implying a severe disease at presentation in our population. The presence of M1, TA (T1 or T2) had a statistically significant correlation with indication for biopsy on univariate analysis, however; only on TA showed a statistically significant correlation on multivariate analysis. There was no statistically significant effect of gender or age on MEST score on multivariate analysis.

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Case Report

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Primary Carcinoid Tumor of Urinary Bladder: A Rare Case Report and Literature Review

Author Affiliation:
*Associate Professor ,**Senior Resident, Department of Urology, Yenepoya Medical College, Derlakatte, Mangalore – 575017 Karnataka.

Reprint Request:
NischithD'souza, Associate Professor, Department of Urology, Yenepoya Medical College, Derlakatte, Mangalore – 575017 Karnataka.
E-mail: nish25@gmail.com

Nischith D'souza*, Ashish Verma, Rahul Bhargava****

Abstract

Carcinoid tumors are known to arise from enterochromaffin cells and are usually found arising from tissue derived from the embryonicneural crest. Although they are more commonly encountered in gastrointestinal and respiratory organs, rarely they have also been encountered in the genitourinary tract, including the kidney and urinary bladder. Only 29 cases of pure carcinoid tumor of bladder have been reported so far in the literature.

We report here another case of pure carcinoid tumor of bladder involving the prostatic urethra and whole of the prostate, who underwent radical cystectomy.

Keywords: Primary Carcinoid Tumor; Bladder Carcinoid; Malignant Bladder Carcinoid; Pure Carcinoid of Bladder.

Introduction

Carcinoid tumors commonly occur in gastrointestinal tract and respiratory tract, but carcinoid tumors of the genitourinary system like kidney, urinary bladder, prostate and urethra have also been reported. About 29 cases of primary bladder carcinoid have been reported so far in the literature [1-3]. Carcinoid tumors arise from enterochromaffin cells and arise from tissue derived from embryonicneural crest.These cells are also known as enterochromaffin cells or amine precursor uptake and decarboxylation (APUD) cells.

There are various theories proposed regarding the origin of these carcinoid tumors like origin frommetaplastic bladder urothelium, or presence of enterochromaffin cells in bladder, or arise from neural crest tissue entrapped within the metanephros during embryogenesis, or that they represent metastases from an occult carcinoid tumor elsewhere in the body [4].

In this report, we describe the clinical, histopathologic, and immunophenotypic features of a pure carcinoid tumor of the urinary bladder and review the literature.

Case Report

A 54 year old male was referred to our out-patient departmentwith a history of haematuria since the past 4 months, with passage of clots and intermittent pain during that period. He was a smoker in the past and had no exposure to aniline dyes. He had no significant medical history. His physical examination was normal. He had already undergone CT-Urogram which showed a polypoidal lesion in the right posterolateral wall involving the right

vesicoureteric junction causing right sided hydroureteronephrosis.Also, he had undergone cystoscopy, which revealed a 4cm lesion in the right posterolateral wall and trigone. Biopsy was taken from that lesion and the histopathological report was transitional cell carcinoma of the urinary bladder(GRADE 3). So, he was referred to our hospital for further management.

In view of these findings, radical cystoprostatectomy and dissection of internal iliac group of nodes with ileal conduit was done.The histopathology report was as follows:

- Grossly, it wasulceroproliferative growth in the lower wall of the bladder close to the prostatic urethra measuring 3x2 cms. Tumourwas infiltrating into the prostatic urethra and prostate. There was diffuse thickening of entire wall of bladder more in the lower part. (Figure 1A).
- Microscopically,tumor showed ulcerated mucosa with tumor cells arranged in nesting pattern. Tumor cells were small, monotonous and round, with round, bland nuclei with stippled chromatin and indistinct nucleoli. The nests were separated by septae of vascular tissue (Figure 1B, 1C). Tumor showed muscle invasion and serosa, vascular and perineural invasion. Also, the whole of the prostate was replaced by tumor cells and the tumor extended into the prostatic urethra (Figure 2D). And there was involvement of iliac group of lymphnodes with vessels filled with tumor emboli (Figure 2C).
- Immunohistochemistry study was strongly positive for Neuron Specific Enolase (NSE) (Figure 2B) and Cytokeratin (Figure 2A).

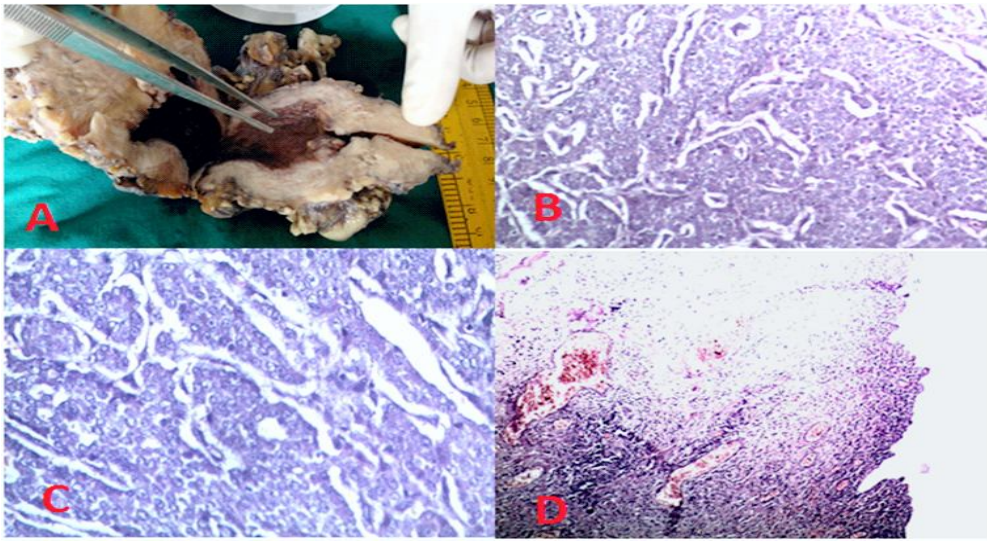

Fig. 1: A–Radical cystectomy specimen cut open to reveal the tumor, B- Monotonous cells arranged in nesting pattern separated by fibrous septae (40X), C- High power view (100X), D- Cystitis with ulceration.

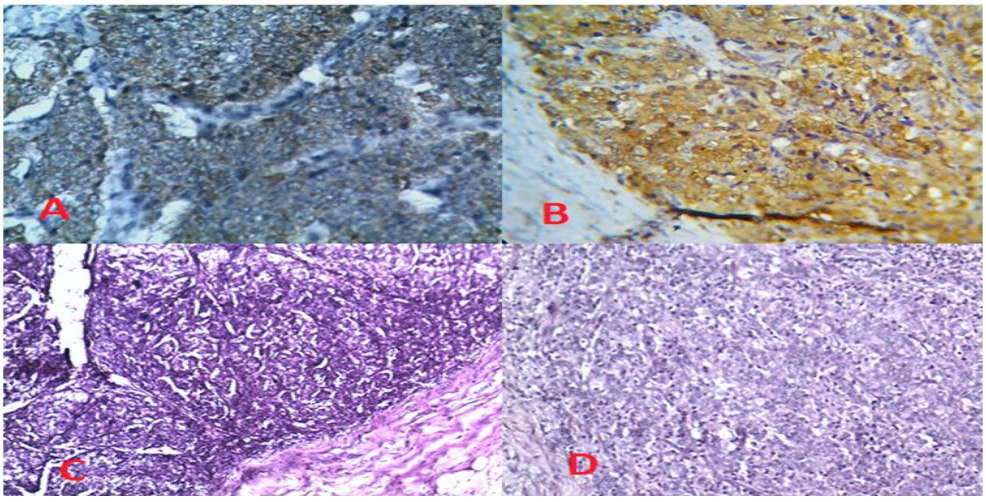

Fig. 2: A-Cytokeratin expression in the tumor, B- Neuron specific enolase expression in the tumor, C-Lymph node showing secondaries, D- Prostatic urethra showing tumor invasion

Thus, it was a carcinoid tumor of the bladder. And as there was no evidence of carcinoid tumors in any other organs, we concluded that this was a case of primary carcinoid tumor of the bladder with invasive nature. There was no element of transitional cell carcinoma found in the specimen.

Discussion

Neuroendocrine tumorscomprise of 1.7% of all bladder tumors [1]. As per the Travis classification, they can be classified into low-grade carcinoid tumors, and high-grade small-cell and large-cell carcinomas [2]. Among these, small cell carcinomas are the most common, with only 29 cases of pure bladder carcinoids reported so far in the literature.

Carcinoid tumors have a variety of growth patterns. On the basis of architecture, they can be classified as insular, trabecular, glandular, undifferentiated, and mixed [5]. Primary bladder carcinoid lesions are mostly seen over trigone or bladder neck and can coexist with other malignancies of bladder as well as inflammatory diseases of the bladder. Usually carcinoid tumours are present submucosally and on cystoscopy appear as polypoid lesions. In all reported cases neuroendocrine differentiation has been readily confirmed by presence of argyrophilic granules or by immunohistochemistry, which reveals presence of chromogranin, synaptophysin, neuron-specificenolaseor other markers of neuroendocrine differentiation.Though carcinoids are slow growing tumors, about 75% of patients have local, distant and / or nodal metastasisat the time of diagnosis [4,6]. Our patient too, had tumor metastasis to the prostate, prostatic urethra and iliac lymph nodes.

As these are very rare tumors, much is not known about their progression and response to various treatment modalities or their long term management.For localizedcarcinoid tumors of the urinary bladder, a variety of treatments have been tried: a multiplebiopsy, transurethral resection, a partial cystectomy, anda total cystectomy or radical cystoprostatectomy depending upon the size of tumor and extent of disease. Many of the reported tumors were small and so were cured by transurethral resection [1].

Not much is known about treatment for metastatic disease, owing to rarity of these tumors. Based upon the studies on neuroendocrine tumors in other parts of the body, two chemotherapy regimens have been recommended: methotrexate, vinblastine, adriamycin, and cisplatin (MVAC) formixed type and cisplatin-etoposide for pure neuroendocrine tumors [7]. But theoverall prognosis of bladder neuroendocrine tumorsremains poor, with a 2-year survival rate of 13%, mostly based on a series ofsmall-cell carcinomas [7].

In view of lack of long term follow-up studies for bladder carcinoids as a consequence of paucity of pure carcinoids cases, the behaviour of these tumors is largely unknown and difficult to predict. Though, size and extent of the lesions usually appear to be most important, and also flow cytometric analysis of DNA ploidy may provide additional prognostic information [8],but nothing can be said conclusively. Mixed carcinoid tumors usually exhibit more aggressive behavior, in line with that expected of the noncarcinoid component [4].

In bladder carcinoids, carcinoid syndrome is not seen due to the fact that the peptides are flushed out of the system in the urine and are not absorbed in the systemic circulation in enough quantities so as to cause symptoms, exception being in cases of metastasis giving rise to large tumour burden, extensive nodal metastasis, or tumours with direct access to systemic circulation.

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[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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