DISSERTATION ON

A STUDY OF ANEMIA IN POST RENAL TRANSPLANT PATIENTS

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CERTIFICATE

This is to certify that dissertation entitled "**A STUDY OF ANEMIA IN POST RENAL TRANSPLANT PATIENTS"** is a bonafide work done by **DR.G. GEETHA,** post graduate student, Institute of Internal Medicine, Madras Medical College, Chennai – 3 in partial fulfillment of the University Rules and Regulations for the award of MD Branch – I General Medicine, under our guidance and supervision, during the Academic period from March 2008 to April 2011.

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I solemnly declare that the dissertation entitled **"A STUDY OF ANEMIA IN POST RENAL TRANSPLANT PATIENTS"** is done by me at Madras Medical College, Chennai – 3 during March 2008 – April 2011 bunder the guidance and supervision of Prof.P.CHITRAMBALAM,M.D., to be submitted to The Tamilnadu Dr.M.G.R. Medical University towards the partial fulfillment of requirements for the award of M.D. DEGRE IN GENERAL MEDICINE BRANCH – I.

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ABBREVIATIONS

ACD	-	Anemia in Chronic disease
AKI	-	Acute kidney injury
CD	-	Cluster differentiation
CGN	-	Chronic glomerular nephritis
CIN	-	Chronic interstitial nephritis
CKD	-	Chronic Kidney diseases
CKDT	-	Transplant Chronic Kidney disease stage
CMV	-	Cytomegalovirus
CRP	-	C-reactive protein
CVD	-	Cardio Vascular diseases
DM	-	Diabetes mellitus
EPO	-	Erythopoietan
ESAS	-	Erythropoiesis Stimulating Agents
ESRD	-	End Stage Renal Disease
FPN	-	Ferroportin
PTA	-	Post Transplant Anema
GFR	-	Glomerular Filtration Rate
HB	-	Hemoglobin
HHV	-	Human Herpes Virus

hsCRP	-	High sensitive C- Reactive protein
HUS	-	Hemolytic uremic syndrome
IL6	-	Interleukin 6
ISD	-	Immunosuppressive drugs
LDH	-	Lactate dehydrogense
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration
MCV	-	Mean Corpuscular Volume
NAAC	-	National Anemia Action Council
PCV	-	Packed Cell Volume
PRCA	-	Pure Red Cell Aplasia
PSS	-	Peripheral smear study
RPI	-	Reticulocyte Production Index
RR	-	Relative Risk
SHT	-	Systemic hypertension
TI	-	Total Iron Binding Capacity
TSAT	-	Transferrin Saturation
TRESAM	-	Transplant European Survey on Anemia management
TTP	-	Thrombotic Thrombocytopenic Purpura

CONTENTS

INTRODUCTION

INTRODUCTION

Anemia of chronic kidney disease is corrected by restored erythropoietin, synthesized from the graft and is generally corrected by three months after successful kidney transplantation.

However, it was seen that, a significant number of recipients continued to be anemic in the post transplant period. Data on the prevalence of post transplant anemia are limited.

The role of immunosuppressive drugs in causation as well as aggravation of anemia is well known and although contributory, Our study aims to enumerate factors other than drugs, which might contribute to the persistence of anemia in the post transplant period, particularly erythtopoietin levels in relation to the hemoglobin levels and reticulocyte counts.

AIMS AND OBJECTIVES

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To asses the incidence of anemia in post renal transplant patients with successful engraftment, and evaluate the possible factors contributing to an inappropriate erythropoietin response.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

CHRONIC KIDNEY DISEASE

Patients with CKD most often present with nonspecific complaints or are asymptomatic and are referred to a nephrologist because of abnormal blood or urine findings. As with AKI, it is important to establish the cause of CKD. Once this has been accomplished, further evaluation may be important in order to maximize the potential to preserve or restore glomerular filtration rate (GFR). Evaluation methods for CKD are similar to those for AKI; however, specific evaluations based on cause and chronicity are essential in patients with CKD. In particular, evaluation of cardiovascular risk factors is critical because of the high rate of cardiovascular complications in CKD.

TABLE Risk Factors for Chronic Kidney Disease

Established risk factors
Age
Gender (male predilection)
Race (African American, Hispanic, Native American)
High blood pressure
Diabetes mellitus
Obesity
Metabolic syndrome
Proteinuria
Family history of kidney disease
Smoking
Atherosclerosis
Exposure to nephrotoxins such as analgesics, heavy metals
Dyslipidemia
Reduced nephron number at birth
Recurrent urinary tract infection

Emerging risk factors
Oxidative stress
Elevated plasma homocysteine level
Anemia
Prothrombotic factors (e.g., plasminogen inhibitor activator-1)

Prevalence of Chronic Kidney Disease

It is estimated that approximately 20 million Americans have CKD[1]. Both the prevalence and the incidence of CKD are increasing. The most common causes of CKD leading to ESRD are diabetes mellitus, hypertension, glomerulonephritis, and cystic kidney disease, which together account for 90% of all new cases of CKD. In the United States, estimates of prevalence of CKD[2] vary from 10 to 20 million, depending on the definition. Confusion in terminology as well as methods for defining CKD and its severity have hampered our ability to identify patients and, therefore, how to approach the problem of CKD. The Kidney Disease Outcomes Quality Initiative (K/DOQI)[5] clinical practice guidelines for CKD evaluation, classification, and stratification published by the National Kidney Foundation provide a framework designed to

address the growing burden of CKD in the United States. The guidelines emphasize the need for prevention, early diagnosis, and treatment of CKD. Similar data in Indian population currently limited.

Definition and Staging of Chronic Kidney Disease

CKD is defined as kidney damage with or without decreased GFR, manifested as either pathologic abnormalities or markers of kidney damage, including abnormalities in composition of blood or urine, abnormality renal imaging findings, and a GFR less than 60 mL/min 1.73 m^2. This broad definition includes patients with or without symptoms of kidney disease.

Staging of Chronic Kidney Disease[6]

Stage	Description	Estimated GFR[*]	Evaluation Plan
	At increased risk	>90 (with CKD risk factors)	Screening CKD risk reduction
1	Kidney damage with normal or increased GFR	≥90	Diagnose and treat cause, slow progression, evaluate risk of cardiovascular disease
2	Kidney damage with mild decrease in GFR	60–89	Estimate progression
3	Moderate decrease in GFR	IIIa 45-59 IIIb 30-44	Evaluate and treat complications
4	Severe decrease in GFR	15–29	Prepare for renal replacement therapy
5	Kidney failure	<15	Initiate renal replacement therapy

ESTIMATION OF GLOMERULAR FILTRATION RATE

TABLE Formulae for Estimating Glomerular Filtration Rate Using Serum Creatinine and Other Clinical Parameters[7]

Formula	Units	Reference
(100/*Cr*) - 12 *if male*	ml/min/1.73 m^2	Jelliffe
(80/*Cr*) - 7 *if female*		
(*Wt* · (29.3 - 0.203 · *Age*)/(*Cr* · 14.4), *if male*	ml/min	Mawer
(*Wt* · (25.3 - 0.175 · *Age*)/(*Cr* · 14.4), *if female*		
(98 - 16 · (*Age* - 20)/20)/*Cr*, *multiply by* 0.90 *if female*	ml/min/1.73 m^2	Jelliffe
((140 - *Age*) · (*Wt*))/(72 · *Cr*), *multiply by* 0.85 *if female*	ml/min	Cockcroft and Gault
((145 - *Age*)/*Cr*) - 3, *multiply by* 0.85 *if female*	ml/min/70 kg	Hull
(27 - (0.173 · *Age*))/*Cr*, *if male*	ml/min	Bjornsson
(27 - (0.175 · *Age*))/*Cr*, *if female*		

Formula	Units	Reference
$7.58/(Cr \cdot 0.0884) - 0.103 \cdot Age + 0.096 \cdot Wt - 6.66$, *if male*	ml/min/1.73 m^2 (height^2)	Walser
$6.05/(Cr \cdot 0.0884) - 0.080 \cdot Age + 0.080 \cdot Wt - 4.81$, *if female*		
$170 \cdot Cr^{-.999} \cdot Age^{-.176} \cdot (0.762$ *if female*$) \cdot (1.180$ *if black*$) \cdot SUN^{-.170} \cdot Alb^{.318}$	ml/min/1.73 m^2	Levey

Alb, serum albumin (g/dL); Cr, serum creatinine (mg/dL); SUN, serum urea nitrogen (mg/dL); Wt, body weight (kg).

MDRD Formula (Modification of Diet in Renal disease)

GFR (ML perminute per 1.73m^2) = 186 x $(SCR)^{-1.154}$ x $(age)^{-0.203}$

x (0.742 if female)

x (1.210 if African – American)

DEFINITION AND PREVALENCE OF ANEMIA IN CHRONIC KIDNEY DISEASE

Anemia is a state of deficient mass of red blood cells and hemoglobin (Hb), resulting in insufficient oxygen delivery to the body's tissues and organs. The normal values for Hgb and hematocrit (Hct) depend on gender, race, and other factors. In addition, the reference range changes in older individuals, with an unexpected increase in the prevalence of anemia. The National Kidney Foundation's clinical practice guidelines define anemia[12] as a Hgb level less than 13.5 g/dL for adult men and less than 12.0 g/dL for adult women. For patients living at altitude, a greater red cell mass is required to maintain tissue oxygen delivery given the reduced ambient oxygen tension. In addition, permanent residents at high altitude develop reduced Hgb oxygen affinity.

TRANSPLANTATION

In general, patients who are not clinically uremic should not be transplanted until the estimated glomerular filtration rate (eGFR) is ≤15 mL/min or less or at least 20 mL/min[13]. In the United States, patients can begin accruing waiting time (which most strongly determines when

patients will be allocated a deceased donor kidney) only when the eGFR is 20 mL/min or less. The rate of progression is also an important consideration, however. Patient with diabetes, for example, may progress relatively rapidly, and when it is clear that the patient will require renal replacement therapy within the next few months, there is no sense in delaying transplantation if a living donor is available.

Absolute Contraindications

Most absolute contraindications to kidney transplantation are not irreversible contraindications. Absolute contraindications include life-threatening infections, cancer, unstable cardiovascular disease (CVD), and noncompliance. However, infections can often be effectively treated, cancers can be cured, organs threatened by occlusive arterial disease can be protected with revascularization procedures, and noncompliant patients can become compliant. Nevertheless, not every patient with stage 5 chronic kidney disease (CKD) is a transplant candidate.[14]

In general, patients who would not be expected to survive more than 2 years with a kidney transplant should probably not undergo transplantation. If such a patient has a potential living donor, it may not be ethical to subject that donor to the risk of surgery when expected

outcomes are poor. If such a patient does not have a potential living donor, he or she will be unlikely to survive long enough on the waiting list to receive a deceased donor kidney. The argument can also be made that a deceased donor kidney may result in greater benefit if it is allocated to a patient with a life expectancy greater than 2 years than if it is allocated to someone who is not expected to live that long. Physicians and transplant centers should inform patients when they are not transplant candidates, and they should not give the patients false hopes or expectations. It is not a good practice to place a patient on the waiting list who is not a good candidate, assuming that the patient will not survive long enough to be offered a kidney and/or intending to refuse an offered kidney if it is made available.

TABLE Transplant Candidate Evaluation to Minimize Infection Risk[15]

Infection	Screening	Action
HIV	HIV antibody	Transplant only if stringent conditions are satisfied
Tuberculosis	PPD, chest x-ray	Consider prophylaxis
Influenza	None	Vaccinate yearly

Infection	Screening	Action
Pneumococcus	None	Vaccinate at least every 5 yr
HBV	HBsAg, HBsAb	Evaluate further if HBsAg positive, otherwise vaccinate to achieve and sustain a HBsAb response
HCV	HCV antibody	Evaluate further if HCV positive
CMV	CMV antibody	Post-transplant prophylaxis
VZV	VZV	Vaccinate if antibody negative
Childhood infections	None	Measles, mumps, rubella, etc.

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PPD, purified protein derivative; VZV, varicella zoster virus.

ANAEMIA IN RENAL TRANSPLANT PATIENTS

Anemia is relatively common after transplantation. Regular screening and careful evaluation of the multiple factors that can contribute to anemia after transplantation are recommended.

Prevalence of anemia[33] in transplant CKD clearly is associated with the level of allograft function. However, a number of other factors unique to transplant recipients may contribute to the development of PTA. There is little information regarding the association of anemia with either graft or patient outcomes. Similarly, there is limited information to suggest that the response to treatment with ESAs differs between patients with CKD with and without a transplant. Although there are reports of increased delayed graft function and hypertension with the use of ESAs[34], there is insufficient evidence to suggest that these agents should not be used in either the immediate posttransplantation or late posttransplantation period. Conversely, with the existing information, it is recommended that treatment guidelines for management of anemia in the general CKD population be followed in the transplant population.

Transplant recipients are a subgroup of patients with CKD with unique considerations with regard to anemia management.

Transplant recipients have variable exposure to CKD before transplantation. Patients with functioning transplants include preemptive transplant recipients, recipients with variable exposure to dialysis, and repeat and multiorgan transplant recipients. Consequently, the burden of comorbid disease varies among transplant recipients, and this may have important implications for the severity and management of anemia.

PREVALENCE

There is no accepted definition of anemia in transplant recipients, and variable definitions are used in the literature. A second important consideration is that the prevalence of anemia is dependent on the time of observation after transplantation.

During the early post transplantation period, arbitrarily defined as the first 6 months after transplantation, anemia of varying degrees is very common. The prevalence[35] and degree of anemia during this period are dependent on the pre transplantation Hb level, amount of perioperative blood loss, frequency of blood draws, iron depletion, persistence of uremia, endogenous erythropoietin levels, erythropoietin responsiveness, and exposure to immunosuppressive agents.

The time course of erythropoiesis after transplantation has been studied by a number of investigators, and reviews on this subject are available. A transient early peak of erythropoietin is detectable within the first 24 hours after transplantation, particularly in patients with delayed graft function, and is not associated with a measurable increase in Hb level. Within the first number of days after successful transplantation, a smaller, more sustained erythropoietin peak is detectable. This peak is associated with the subsequent onset of erythropoiesis and recovery of renal anemia during the next several months after transplantation.

(Ref : K/DOQI clinical
practical guide lines for ckd)

ERYTHROPOIESIS AND PRODUCTION OF EPO BY THE RENAL ALLOGRAFT

Successful renal transplantation results in the normalization of renal function and resolution of anemia of CKD. In contrast to non renal forms of anemia in which serum EPO levels are inversely correlated with the levels of Hb, with anemia of renal disease, the lower the EPO levels, the worse the anemia. Therefore, the early resolution of anemia in post transplantation is because of the normal mechanisms that regulate the production and secretion of EPO. The events that result in the normalization of erythropoiesis and the early recovery of anemia can be summarized as follows:

1. An early rise in EPO secretion starts within the first 24 hours after transplantation[43 44]. This initial peak is transient and ineffective in generating normal eruthropoiesis.

2. After the first week post transplant, a smaller and more sustained peak of EPO production occurs. This second peak is associated with the subsequent onset of erythropoiesis and recovery of anemia over the next 2 to 3 months [43 44]

3. By 10 weeks after transplant, serum EPO concentrations should reach 100% of the expected levels in individuals with normal functioning grafts.

PATHOPHYSIOLOGY

A number of factors may cause PTA; some are shared with other patients with CKD, whereas others are unique to transplant recipients.

Factors Shared With Other Patients With CKD

In general, the evaluation of PTA should parallel that among non transplantation patients with CKD. The discussion regarding factors contributing to PTA that are shared with other patients with CKD is limited to the most common considerations and to unique considerations in the transplantation setting.

Kidney Function

Level of allograft function is clearly an important determinant of PTA. In the TRESAM (Transplant European Survey on Anemia Management, there was a strong association of anemia with kidney transplant function. Of 904 patients with an S_{Cr} level greater than 2 mg/dL, 60.1% were anemic compared with 29% of those with an S_{Cr} less

than 2 mg/dL, In a single-center study of 459 patients at least 6 months after transplantation, prevalences of anemia, defined as an Hb level less than 11 g/dL, among patients with CKD stages 1, 2, 3, 4, and 5 were 0%, 2.9%, 6.6%, 27%, and 33%, respectively.

The association of level of allograft function with Hb level appears to vary with the time of observation after transplantation. One study found that, among patients with an eGFR greater than 90 mL/min/1.73 m^2, 11% and 7% of patients had anemia at 6 and 12 months after transplantation, whereas among patients with an eGFR less than 30 mL/min/1.73 m^2, at 6 and 12 months after transplantation, 60% and 76% were anemic, respectively. These findings suggest that factors in addition to level of allograft function may be important determinants of anemia, particularly during the early post transplantation period.

Whether the association between anemia and level of kidney function differs in patients with CKD who did and did not undergo transplantation is uncertain. In a study of 23 renal transplant recipients with stable kidney function, 12 anemic patients were compared with the 11 non anemic control patients. Of the 12 anemic patients, 10 had low erythropoietin levels suggestive of erythropoietin deficiency, 2 patients had higher than anticipated erythropoietin levels suggestive of

erythropoietin resistance, whereas 5 of 11 non anemic control patients had higher than expected erythropoietin levels. Thus, there appears to be significant variation in erythropoietin production and responsiveness in transplant recipients, which may alter the association between kidney function and anemia in transplant recipients. Other clinically evident examples of the dissociation between Hb level and kidney function in transplant recipients include posttransplantation erythrocytosis and, as in non transplantation patients with CKD, the lower incidence of anemia among transplant recipients with polycystic kidney disease.

IRON DEFICIENCY

Iron deficiency may be an important factor in the development of anemia after transplantation. There is limited information regarding the prevalence of iron deficiency after transplantation. In a cross-sectional study of 438 prevalent transplant recipients, the prevalence of iron deficiency, defined[45] as a percentage of hypochromic red blood cells of 2.5% or greater, was 20.1%. In another study[46] of 439 prevalent transplant recipients, 41% of patients had a TSAT less than 20%, whereas 44% had a ferritin level less than 100 ng/mL.

The prevalence of iron deficiency may be greater in the early transplantation period because of low pre transplantation iron stores in dialysis patients and increased iron utilization with the onset of erythropoiesis after successful transplantation. In one study, 24 of 51 patients were found to be iron deficient in the early post transplantation period. In a prospective study of 112 transplant recipients, serum ferritin levels decreased from 109.6 µg/L (range- 21 to 4,420 µg/L) at transplantation to 54.9 µg/L (range- 2 to 1,516 µg/L) at 6 months after transplantation.

Transplant-Specific Factors

Acute Rejection

Early acute rejection is reported to cause a sharp decrease in erythropoietin production and anemia. Insights into the molecular mechanisms involved in the development of anemia during allograft rejection have been elucidated from gene expression studies. Among 4 pediatric renal allograft recipients with acute rejection and anemia, a cluster of 11 genes involved in Hb transcription and synthesis, iron and folate binding, and transport were found to be downregulated. An additional mechanism for the development of anemia during rejection is thrombotic microangiopathy, which may develop during episodes of severe vascular rejection.

MEDICATIONS

IMMUNOSUPPRESSIVE MEDICATIONS.

The use of myelosuppressive medications for immunosuppression and antiviral prophylaxis or treatment may be important factors in the development of anemia after transplantation. Azathioprine and mycophenolate mofetil are myelosuppressive; therefore, anemia caused by these drugs often is associated with leukopenia and/or thrombocytopenia. (Ref – JASN – Anemia after kidney transplantation) Very rarely, PRCA may occur with the use of these drugs.

Calcineurin inhibitors infrequently are associated with anemia. The most common mechanism for PTA associated with the use of calcineurin inhibitors is microangiopathy and hemolysis. The immunosuppressant OKT3 also has been associated with hemolytic uremic syndrome (HUS) and microangiopathy[47].

Anemia was a significant Adverse effect in a phase III trial in which sirolimus was administered with cyclosporine and corticosteroids. A group reviewed the 10-year experience with sirolimus and reported a dose-dependent association of anemia with the drug in phase I and II trials. The association of sirolimus with anemia also

recently was shown in single-center analyses. Sirolimus may inhibit erythropoiesis by interfering with intracellular signaling pathways normally activated after the binding of erythropoietin to its receptor, and sirolimus also may be associated with thrombotic microangiopathy.

ANTIVIRAL AND ANTIMICROBIAL MEDICATIONS.

A number of commonly used antivirals and antibiotics may cause anemia, including ganciclovir and trimethoprim-sulfamethoxazole.

Angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists.

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) may be associated with PTA[48]. Anemic patients in the TRESAM had higher odds of receiving ACE inhibitors or ARBs (odds ratio, 1.55; 95% CI, 1.34 to 1.80; $P < 0.001$). In a single-center retrospective study, a significant curvilinear dose-response relationship was identified between ACE-inhibitor dose and Hct. The underlying mechanisms are complex and include inhibition of endogenous erythropoietin production, inhibition of angiotensin II–mediated stimulation of red blood cell precursors, and the generation of an erythropoiesis-inhibiting protein by ACE inhibitors.

INFECTIONS AND MALIGNANCY

Anemia may be a feature of cytomegalovirus (CMV) infection. Parvovirus B19 infection has been reported in transplant recipients with anemia and may cause PRCA[49].

Hemophagocytic syndrome (HPS) is a rare cause of PTA. The syndrome often is caused by infectious or neoplastic disease and is defined by bone marrow and organ infiltration by activated non-malignant macrophages that phagocytose red blood cells. A retrospective analysis of 17 cases among deceased donor transplant recipients showed that the syndrome developed after a median duration of 52 days after transplantation. Fever was present in all patients and hepatosplenomegaly was present in 9 of 17 patients. Eleven patients had received anti lymphocyte globulins in the 3 months before presentation. In 9 patients, HPS was related to viral infections (CMV, Epstein-Barr virus, and human heprus virus 6 and 8); other infections included tuberculosis, toxoplasmosis, and *Pneumocystis carinii* pneumoniae. Post transplantation lymphoproliferative disease was present in 2 patients. The syndrome has a poor prognosis—8 of 17 patients died despite the use of anti-infectious therapy and tapering of immunosuppression.

HEMOLYTIC UREMIC SYNDROME

HUS may recur after transplantation and can result in allograft loss. De novo HUS may occur associated with the use of cyclosporine, tacrolimus, or OKT3. The syndrome also has been associated with CMV and influenza A infection in transplant recipients. The possibility that erythropoietic agents may be beneficial in patients with HUS after transplantation is suggested by observations from the non transplantation population. Plasma from approximately 75% of patients with sporadic thrombotic thrombocytopenic purpura (TTP)/HUS induces apoptosis in cultured microvascular endothelial cells. The mechanism appears to be linked to induction of Fas (CD95) on cultured endothelial cells because the erythropoietin receptor is expressed on vascular endothelial cells. Erythropoietin prevents lipopolysaccharide-induced apoptosis in cultured endothelial cells, suggesting that erythropoietin may have a protective effect and may be of therapeutic benefit in TTP/HUS in the non transplantation setting. The use of ESAs after transplantation in patients with HUS warrants further study[50].

Hemolytic Anemia Associated With Minor Blood Group A, Group B, Group O Incompatibility

Blood group A recipients receiving transplants from blood group O donors or blood group AB recipients receiving transplants from either group A or B donors may develop evidence of hemolysis caused by anti-A or anti-B antibodies of the donor or from autoantibodies produced by passenger lymphocytes.

CLINICAL OUTCOMES

There is only limited information regarding the association of anemia with clinical outcomes in transplant recipients.

Transplant recipients are known to be at increased risk for cardiovascular events, particularly during the perioperative period. In a single-center study of 404 transplant recipients with diabetes between 1997 and 2000, patients with at least 1 monthly Hct level less than 30% during the first 6 months after transplantation had a significantly greater incidence of cardiovascular events compared with patients with monthly Hct values greater than 30%. In a multivariate analysis that also included patient age and history of ischemic heart disease (IHD), an increase in monthly Hct to greater than 30% was associated with a significantly

lower risk for cardiovascular events (RR, 0.65; 95% CI, 0.33 to 0.91; P = 0.02).

Among long-term transplant recipients, there is limited information regarding the association of anemia with adverse cardiovascular events. In a retrospective study of 638 transplant recipients between 1969 and 1999 who were alive and free of cardiac disease 1 year after transplantation, lower Hb levels were associated with an increased risk for de novo Congestive heart failure (RR, 1.24/10-g/dL decrease in Hb; 95% CI, 1.10 to 1.39; P = 0.001). In a follow-up study, anemia was associated with increase in left ventricular mass (as measured by Cornell voltage on electrocardiogram) during the first 5 years after transplantation.

In a recent study, 438 prevalent transplant recipients were followed up for a median of 7.8 years. Laboratory parameters obtained during a 4-week enrollment period in 1995 were tested for their association with all-cause mortality and allograft failure. The investigators did not identify an association between anemia (Hb < 10 g/dL) and all-cause mortality or graft survival. Compared with patients with hypochromic red blood cells less than 5%, patients with hypochromic red blood cells greater than 10%

had an RR of 2.06 for all-cause mortality (95% CI, 1.12 to 3.79; P = 0.02).

In a retrospective single-center study of resource utilization among 220 kidney transplant recipients, patients with a higher Hct had a decreased risk for hospitalization (RR, 0.95/1% increase in Hct; 95% CI, 0.92 to 0.98; P < 0.001)

Post transplantation Failure

Patients with allograft failure have a high mortality that is related primarily to CVD. The management of anemia among patients with failing allografts is suboptimal. In a study of patients initiating dialysis therapy after transplant failure in the United States between 1995 and 1998, mean Hct was 27.5% ± 5.9% and 67% had an Hct less than 30%. The use of ESAs at the time of dialysis therapy initiation was infrequent (35%). Anemia management may be more difficult in patients with transplant failure because of the presence of chronic inflammation and relative resistance to ESAs.

MATERIALS & METHODS

MATERIALS AND METHODS

Study Design:

Prospective Cohort Study

Study Populations:

Post Renal Transplant Patients with anemia

Inclusion Criteria:

- Hb levels of < 13g/dl – male, < 12g/dl – female
- Patients with minimum of 6 months after transplant ,as the optimum erythropoietin response is observed not earlier than 6 months after transplant. The selected patients didnot have the expected Hb increment 6 months after the transplant [pre-transplant and post-transplant Hb – P value – 0.0002]

		MEAN HB	P-VALUE
PRETRANSPLANT		10.0225	
			0.0002
POST TRANSPLANT		8.8075	

Exclusion Criteria:

Transplant period – < 6 months Graft failure

Ethical Clearance :

Obtained:

Informed Consent:

Obtained from all patients.

Methodology:

A total of 40 patients were observed in the nephrology OPD over a period of 12 months (April 2009 to Mar 2010) according to the above criteria and were included in the study.

A questionnaire prepared, noted the following

- The age at transplantation.
- Likely cause of renal failure
- Comorbid illness

- Transplant related information such as kidney donor, Pretransplant Hb, immunosuppressive drugs used, use of induction therpy, bloodloss, blood transfusion, occurrence of delayed graft function, infection – CMV & TB.

Clinical examination included a detailed examination from head to foot, examination of cardiac, respiratory, GI tract, and nervous systems, per rectal and per vaginal examination (in females) were done.

The above data were procured from every patient included in the study and analysis was made with the following.

- GFR [index of renal function] with the Hb levels
- GFR with serum erythropoietin level
- Erythropoietin with Hb level

The analysis was directed towards evaluating a possible, persisting inflammatory response interfering with erythropoietin regulated hemoglobinization of erythrocytes.

Laboratory Investigations:

Complete blood count, including red cell indices, ESR, peripheral smear study, reticulocyte count,

- Serum iron, TIBC,
- Serum creatinine, GFR,
- CRP, LDH
- Erythropoietin.

Medications other than immunosuppressives such as Gancyclovir, ACE inhibitors, ARBs, use of erythropoietins were recorded.

S.NO	PARAMETER	METHOD
1	Complete blood count	Automated flow cytometry
2	ESR	Westegren method
3	Serum Iron	Photocolorimetric method
4	TIBC	Photocolorimetric method
5	CRP	Latex Agglutination method
6	LDH	UV kinetic methods
7	Erythropoietin	Chemiluminescence Immunassay
8	GFR	Cockcroft gault formula

Statistical Analysis:

Data analysis was done with use of SPSS, version 13. Descriptive statistics were used to calculate the frequency, mean, and standard deviation. To examine the linear trend of the proportions, trend chi-square was used and to find the test of association chi-square was computed.

Financial support:

Nil

Conflicts of interest:

None

NORMAL VALUES

Hemoglobin	M:13.3 – 16.2gms%	F:12.0-15.8gms
PCV	M:38.8 – 46.4	35.4 – 44.4
Total Count	3.54 – 9.06 x 10^3/ mm^3	
Platelet Count	165 – 415 x 10^3 / mm^3	
MCV	80 – 100 fL	
MCHC	32.3 – 35.9g/dL	
MCH	26.7 – 31.9 pg/cell	
ESR	M : 0 – 15 mm/hr	
Reticulocyte count	M : 0.8 – 2.3%	
LDH	115-221U/L	
CRP	0.8 – 3.1 mg/L	
SR.Iron	41-141microg/dl	
TIBC	251-406microg/dl	
SR.Erythropoietin	3.7-29.5microIU/ml	

OBSERVATION & RESULTS

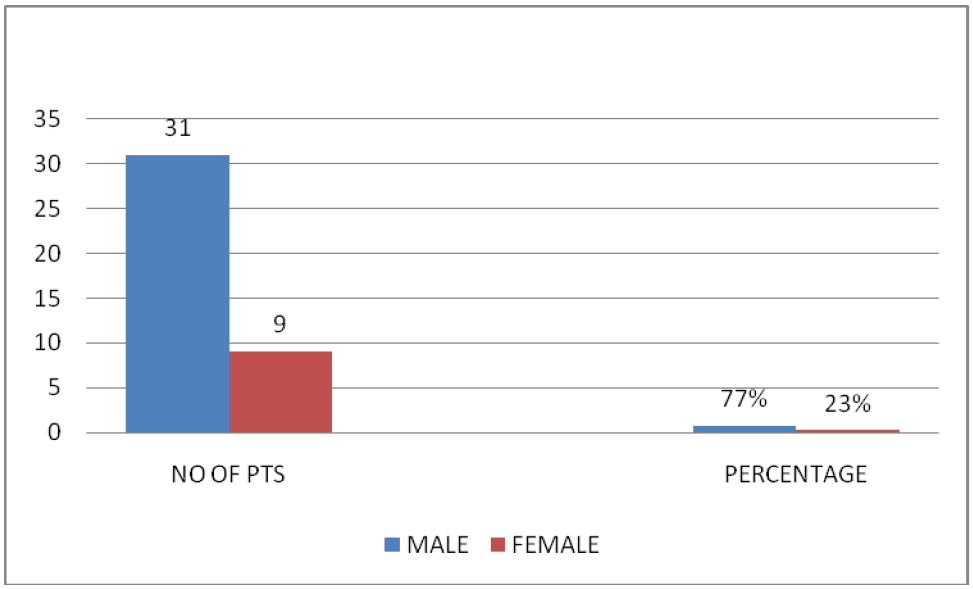
OBSERVATION &RESULTS

STUDY POPULATION CHARACTERISTICS

TABLE 1:SEX DISTRIBUTION

PATIENTS	NO OF PTS	PERCENTAGE	
MALE	31	77%	
FEMALE	9	23%	

FIGURE:1 SEX WISE DISTRIBUTION

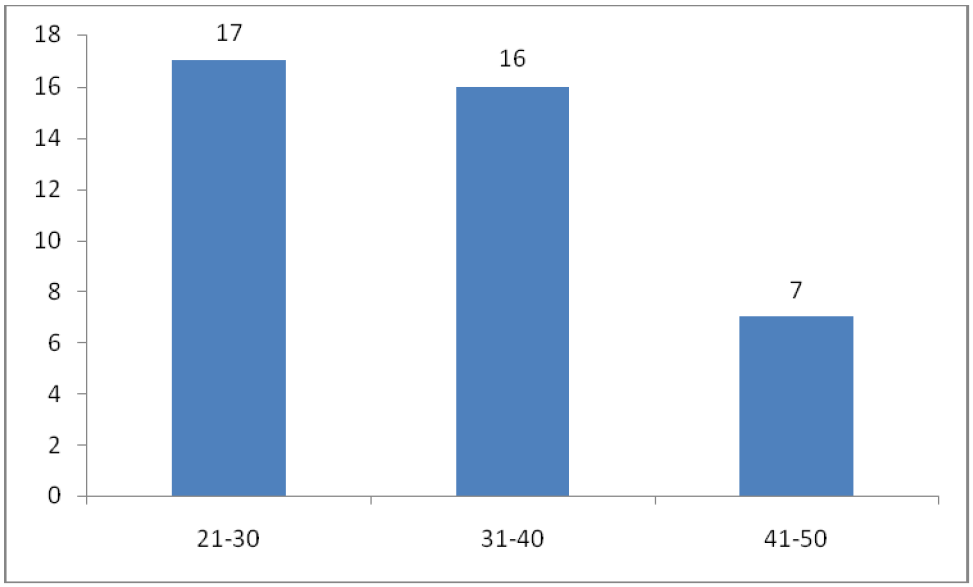


- Our study included a total of 40 patients showing the incidence of male, female ratio

TABLE :2 AGE DISTRIBUTION OF STUDY POPULATION

AGE	NO OF PTS	PERCENTAGE
21-30	17	42.50%
31-40	16	40%
41-50	7	17.50%

FIGURE :2 DISTRIBUTION OF PATIENTS BASED ON AGE

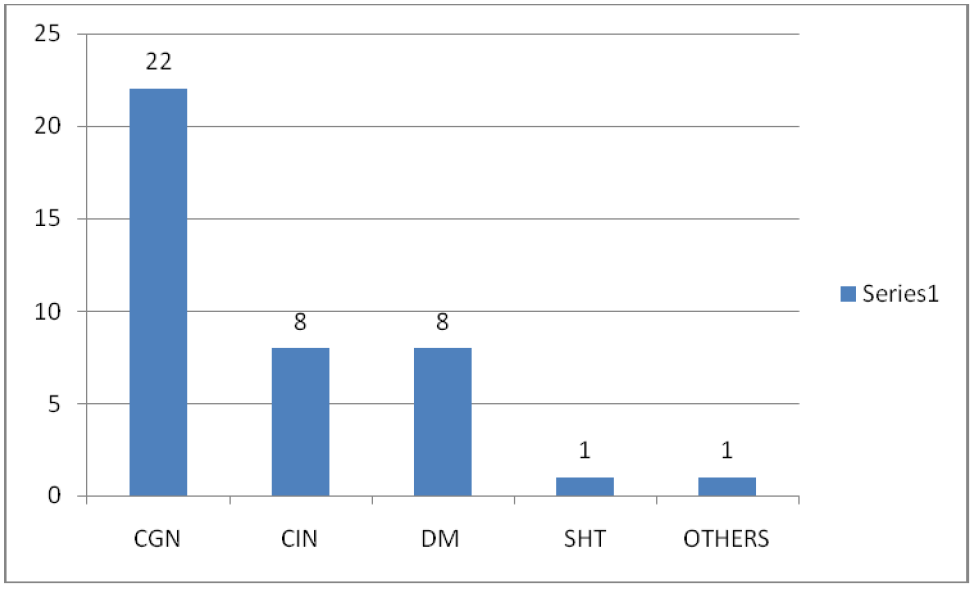


- Patients from 21-50yrs were included in our study with 17 patients in the 21-30yrs[42.5%]
- 16 patients in the 31 -40 yrs[40%] and 7 patients in the 41-50yrs[17.5%]

TABLE-3:DISEASE DISTRIBUTION IN STUDY POPULATION

DISEASES	NO.OF.PTS	PERCENTAGE
CGN	22	55%
CIN	8	20%
DM	8	20%
SHT	1	2.50%
OTHERS	1	2.50%

FIGURE-3: DISEASE DISTRIBUTION IN STUDY POPULATION



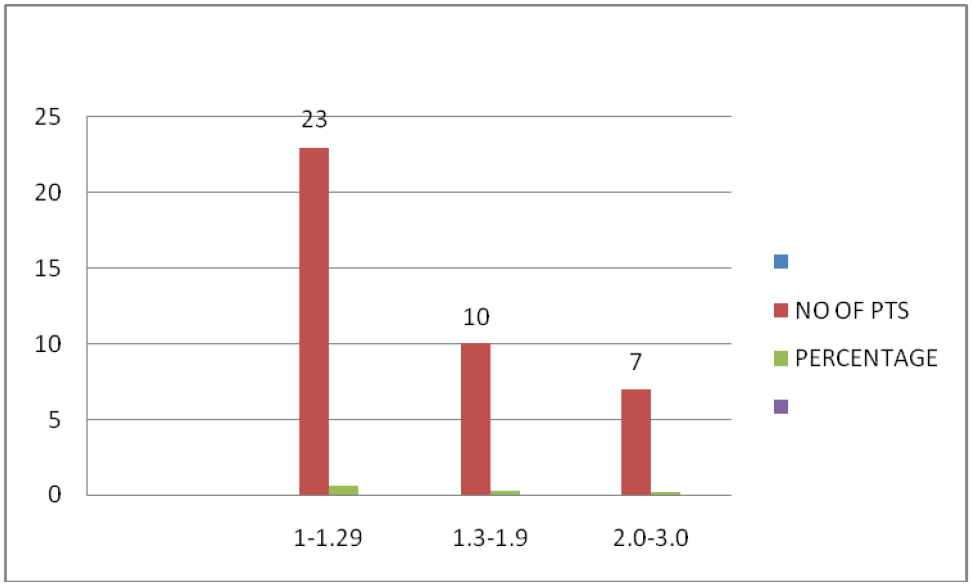
The diseases leading to esrd in the study included

- CGN- 55%
- CIN-20 %
- DM-20%,
- SHT-2.5%,
- OTHERS-2.5%

TABLE-4:SR CREATININE LEVEL IN STUDY POPULATION

SR.CREATININE	NO OF PTS	PERCENTAGE
1-1.29	23	57.50%
1.3-1.9	10	25%
2.0-3.0	7	17.50%

FIGURE 4 : SR CREATININE DISTRIBUTION IN STUDY POPULATION

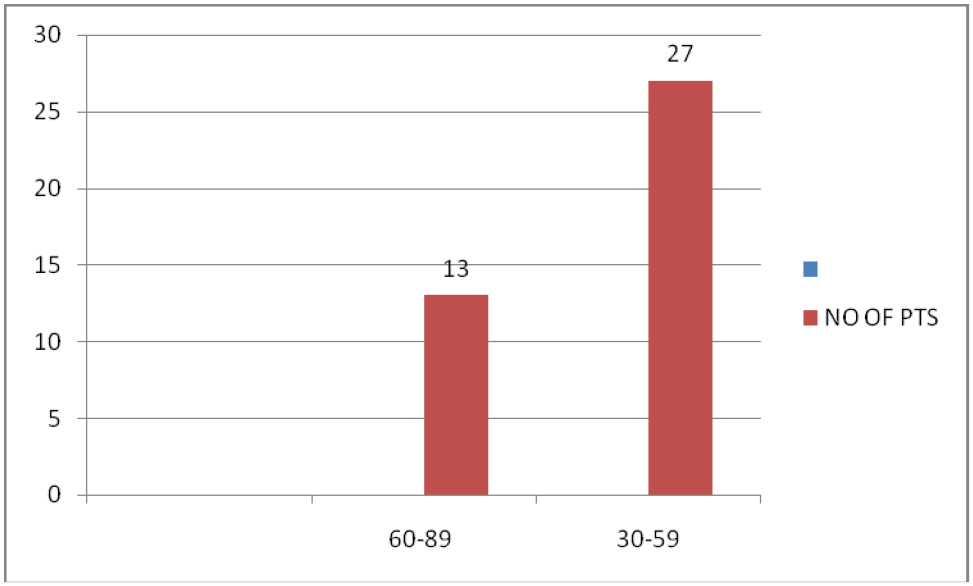


- In our study 23 patients[57.5%] maintained the sr creatinine level within 1-1.29,
- 17 patients [42.5%] maintainingthe sr creatinine level within 1.3-3mg/dl.

TABLE-5:GFR IN STUDY POPULATION

GFR ML/M/1.73 M	NO OF PTS	PERCENTAGE
60-89	13	32.50%
30-59	27	67.50%

FIGURE-5: GFR IN STUDY POPULATION

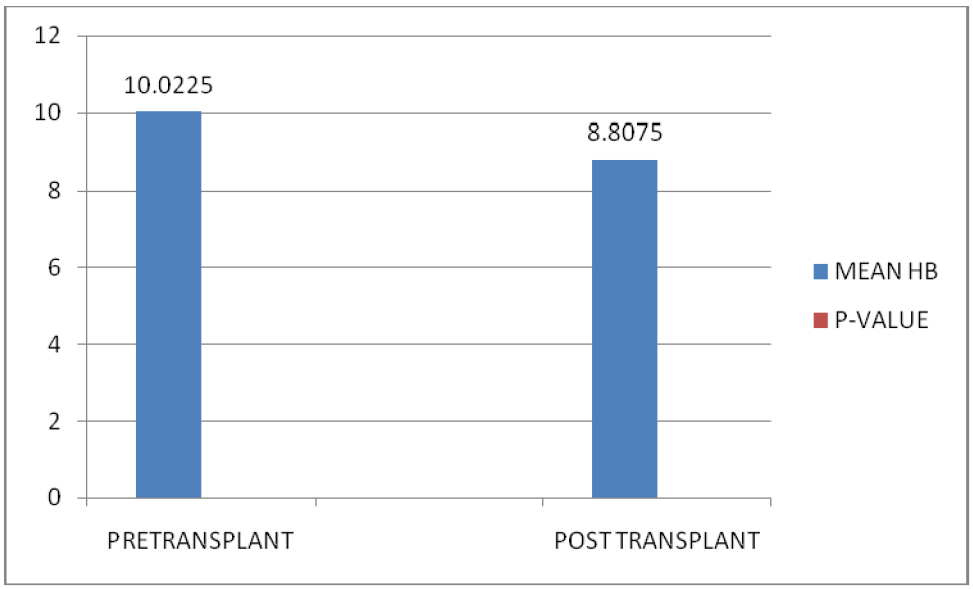


- GFR as a index of renal function indicating that 27 patients [67.5%] were in stage 2 ckdt ,
- 13 patients [32.5%] were in the stage 3 ckd

TABLE-6:HEMOGLOBN IN STUDY POPULATION

	MEAN HB	P-VALUE
PRETRANSPLANT	10.0225	
		0.0002
POST TRANSPLANT	8.8075	

FIGURE-6: HEMOGLOBIN DISTRIBUTION IN STUDY POPULATION

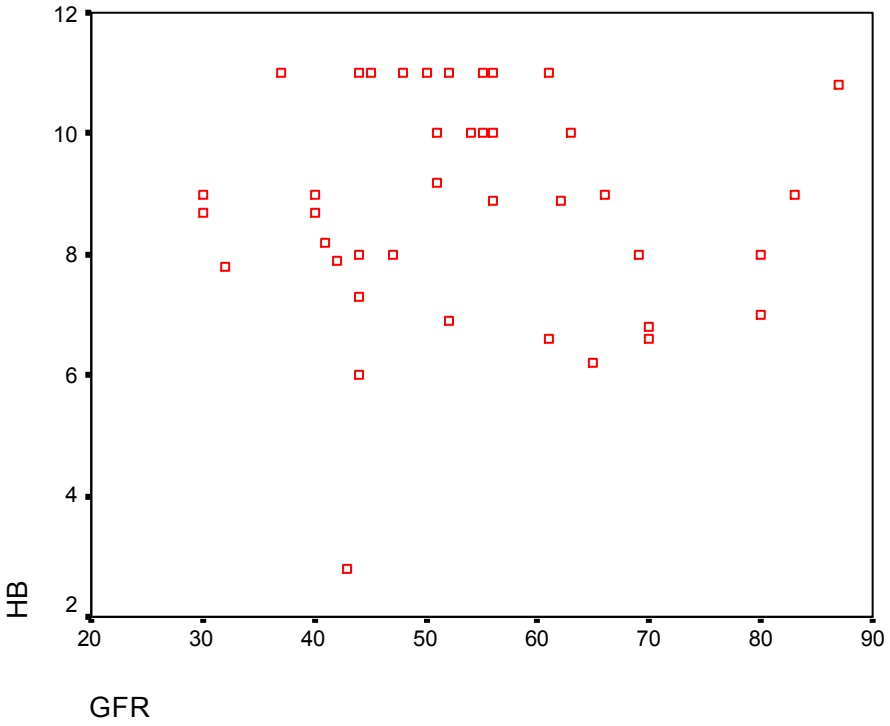


- The patients selected had PRE TRANSPLANT mean Hb of 10.0225gm/dl and post transplant mean Hb of 8.8075 gm/dl with p value of 0.0002, indicating an appropriate patient selection.

TABLE -7: CORRELATION OF HB WITH GFR

Correlation Coefficients Matrix			
Sample size	40	Critical value (5%)	2.0244
		GFR	HB
GFR	Pearson Correlation Coefficient	1	
	R Standard Error		
	T		
	p-value		
	H0 (5%)		
HB	Pearson Correlation Coefficient	-0.0257	1
	R Standard Error	0.0263	
	T	-0.1585	
	p-value	0.8749	
	H0 (5%)	accepted	
R			
Variable vs. Variable	R		
HB vs. GFR	-0.0257		

DISDRIBUTION OF HB ACCORDING TO GFR

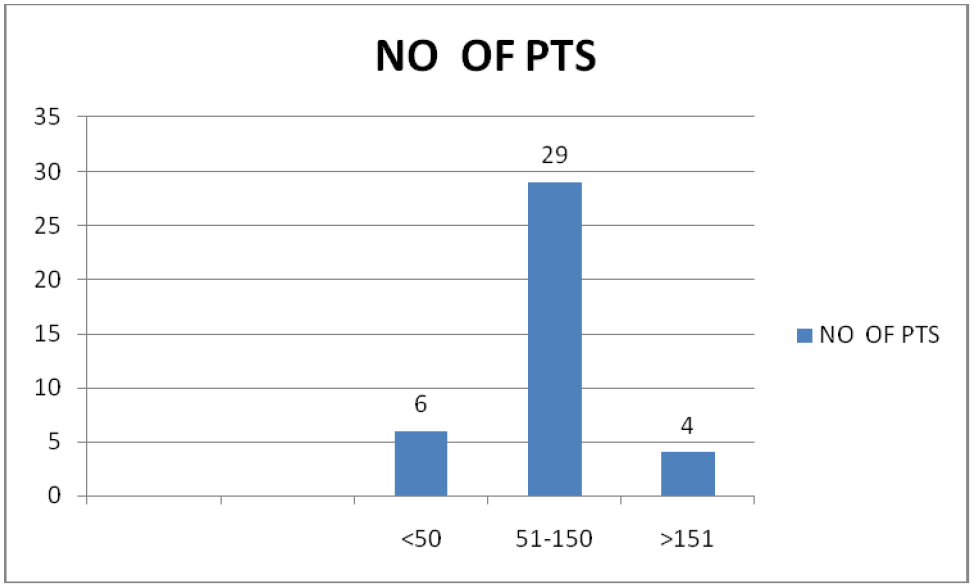


- Hb level of study patients were compared with the GFR [post transplant]. Despite the significant return of renal function [stage2,3ckdt],the expected Hb increment was not noted, with the p value of 0.8749 .

TABLE 8: DISTRIBUTIONOF SR IRON IN STUDY POPULATION

SR IRON	NO OF PTS	PERCENTAGE
<50	6	15%
51-150	29	72.50%
>151	4	10%

FIGURE 8: DISTRIBUTION OF SR IRON IN STUDY POPULATION



- The Serum iron status in 29 patients [72.5%] was within normal range. Only 6 patients had serum iron levels of <50 micro gram/dl[17.5%]. it is probably contributing to the persistence of anemia. About 4 patients [10%] had high serum iron level. In our study, serum iron level didnot correlate with improving GFR.

TABLE 9 : CORRELATION OF IRON WITH GFR

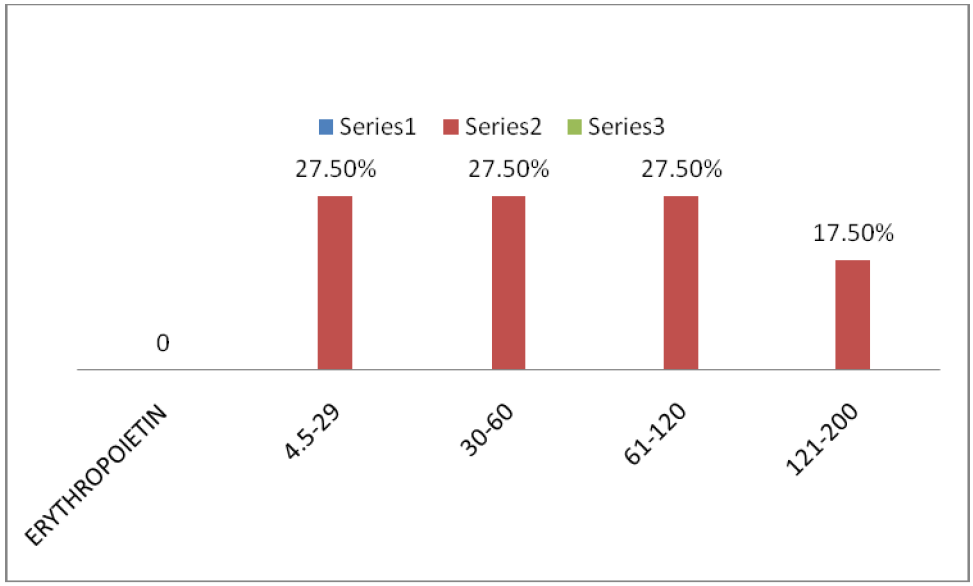
Sample size	**40**	**Critical value (5%)**	**2.0244**
		GFR	**IRON**
GFR	**Pearson Correlation Coefficient**	**1.**	
	R Standard Error		
	t		
	p-value		
	H0 (5%)		
IRON	**Pearson Correlation Coefficient**	**-0.2441**	**1.**
	R Standard Error	0.0247	
	t	-1.5517	
	p-value	0.129	
	H0 (5%)	accepted	
R			
Variable vs. Variable	R		
IRON vs. GFR	-0.2441		

- Iron level of study patients were compared with the GFR [post transplant] with p value of 0.129

TABLE 10 : ERYTHROPOIETIN (ENDOGENOUS) LEVEL IN STUDY POPULATION

ERYTHROPOIETIN	PERCENTAGE
4.5-29	27.50%
30-60	27.50%
61-120	27.50%
121-200	17.50%

FIGURE 10 : ERYTHROPOIETIN (ENDOGENOUS) LEVEL IN STUDY POPULATION



- In our study 27.5% patients had normal erythropoietin level, the others had increased erythropoietin level.

TABLE-11 :CORRELATION OF ERYTHROPOIETIN WITH GFR

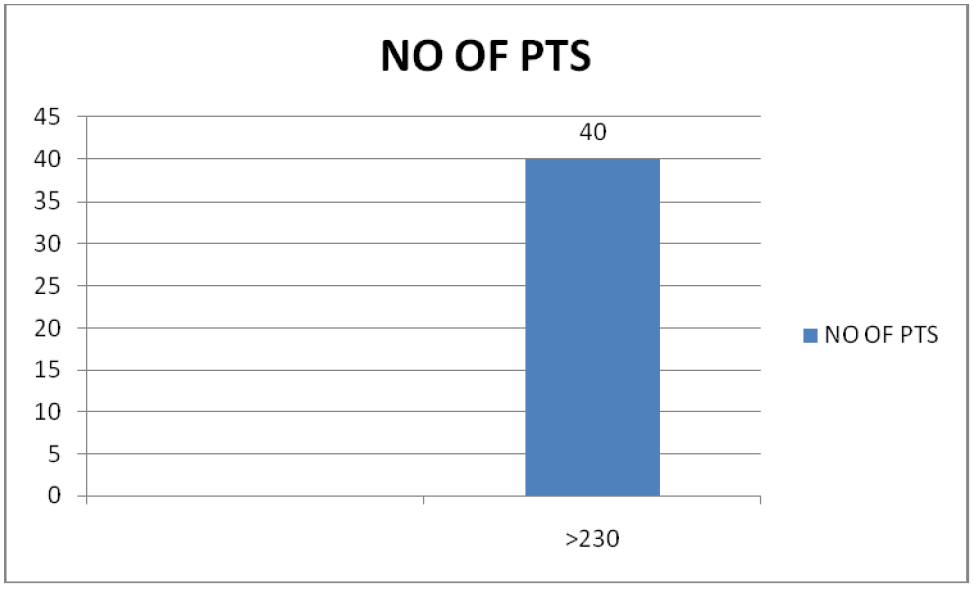
Correlation Coefficients Matrix			
Sample size	40	Critical value (5%)	2.0244
		GFR	EPO
GFR	Pearson Correlation Coefficient	1	
	R Standard Error		
	t		
	p-value		
	H0 (5%)		
EPO	Pearson Correlation Coefficient	0.4277	1
	R Standard Error	0.0215	
	t	2.9172	
	p-value	0.0059	
	H0 (5%)	rejected	
R			
Variable vs. Variable	R		
EPO vs. GFR	0.4277		

- Serum erythropoietin level showed an increase commensurate with return of glomerular function.with the p value of o.0059 which statistically significant.

TABLE 12: DISTRIBUTION OF SERUM LDH IN STUDY POPULATION

SR LDH	NO OF PTS
>230	40

FIGURE 12 : DISTRIBUTION OF SERUM LDH IN STUDY POPULATION



- Sr LDH was above normal in all the 40 patients in our study

TABLE 13 : CORRELATION OF SERUM LDH WITH GFR

Correlation Coefficients Matrix			
Sample size	40	*Critical value (5%)*	2.0244
		GFR	**LDH**
GFR	**Pearson Correlation Coefficient**	**1.**	
	R Standard Error		
	t		
	p-value		
	H0 (5%)		
LDH	**Pearson Correlation Coefficient**	**0.242**	**1.**
	R Standard Error	0.0254	
	t	1.5372	
	p-value	0.1325	
	H0 (5%)	*accepted*	
R			
Variable vs. Variable	*R*		
LDH vs. GFR	0.242		

- There was no correlation of sr LDH value with an improving GFR.with p value of 0.1325.

TABLE 14 : CORRELATION OF SERUM LDH WITH HB

Correlation Coefficients Matrix			
Sample size	39	*Critical value (5%)*	2.0262
		LDH	**HB**
LDH	**Pearson Correlation Coefficient**	**1.**	
	R Standard Error		
	t		
	p-value		
	H0 (5%)		
HB	**Pearson Correlation Coefficient**	**0.0508**	**1.**
	R Standard Error	0.027	
	t	0.3093	
	p-value	0.7589	
	H0 (5%)	*accepted*	
R			
Variable vs. Variable	*R*		
HB vs. LDH	0.0508		

- The high LDH value in response to low Hb could indicate low grade hemolysis, but was not supported by an appropriate reticulocyte response. With the p value of 0.7589 is therefore statistically not significant.

TABLE 15 : CORRELATION OF TIBC WITH GFR

Correlation Coefficients Matrix			
Sample size	40	*Critical value (5%)*	2.0244
		GFR	**TIBC**
GFR	**Pearson Correlation Coefficient**	**1.**	
	R Standard Error		
	t		
	p-value		
	H0 (5%)		
TIBC	**Pearson Correlation Coefficient**	**0.0594**	**1.**
	R Standard Error	0.0262	
	t	0.3667	
	p-value	0.7159	
	H0 (5%)	*accepted*	
R			
Variable vs. Variable	*R*		
TIBC vs. GFR	0.0594		

- TIBC which include ferritin and transferrin, reflects not only percentage saturation but also the response to any underlying chronic inflammation as an acute phase reactant .The TIBC level also didnot show any correlation with improving gfr. With p value of 0.7159 .

TABLE 16 : IRON –TIBC CORRELATION

SR TIBC	NO OF PTS	PERCENTAGE
300-360	4	10%
361-550	10	25%
551-750	10	25%
750-1200	13	40%

90% Patients had significantly high TIBC despite high serum iron levels indicating

- In effective feedback
- Elevated ferritin & transferrin as an acute phase reactant

TABLE 17 : HB -IRON –TIBC CORRELATION

SRNO	HB	SR.IRON	TIBC
1	7.3	120	1012
2	9.5	102	566
3	10	160	108
4	2.8	150	113
5	8	126	690
6	8.2	138	773
7	9.8	191	475
8	10	128	983
9	9.6	135	968
10	12	161	953
11	10	105	743
12	9	116	905
13	9	111	614
14	10	163	724
15	11	130	565

- From the above table it is evident that high SR TIBClevel persisted in patients with high serum iron levels. Among the 12 patients, 6 [50%] of them continue to have low Hb level <10 gm%, The SERUM IRON, TIBC levels reflecting the pattern observed in anemia of chronic disease.

TABLE 18 : HB -MCV –IRON CORRELATION

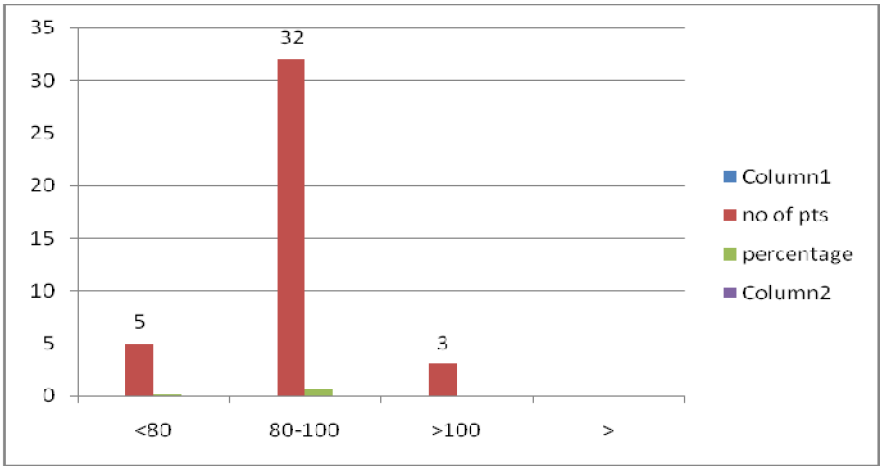
SR NO	HB	MCV	SR IRON
1	6.9	75	160
2	11	79	71
3	8	74	42.7
4	7	72	70
5	11	78	128

- Correlation between HB/MCV/SR IRON was analysed it was found that in patient with microcytosis [mcv<80],sr iron significantly elevated in 2 of 5 patients [40%] indicating alternate mechanism of ineffective erythropoiesis.

TABLE 19 : MCV LEVEL IN STUDY POPULATION

mcv	CHARECTERSTICS	no of pts	percentage
<80	microcytic	5	12.50%
80-100	normocytic	32	80%
>100	macrocytic	3	7.50%

FIGURE 19 : MCV LEVEL IN STUDY POPULATION



- Mcv analysis in the 40 patients showed Microcytosis in [12.5%] , Normocytosis in [80%] , Macrocytosis in [7.5%]

- 32 patients had normocytic anemia ;out of this 17 had CGN, 7 patient had CIN.7 patient had DM 1 patient had SHT [25 -62.5%],

- In Patients with CGN,DM ,SHT the basic pathology may still play a role in the post transplant period, inciting a renal compromise and erythropoietin resistance.

- CRP –The c reactive protein value of 40 patient included in our study was normal, however, the sensitivity of CRP being low ,this particular parameter could not be taken into consideration for analysis. hs CRP being a more sensitive parameter would be of greater significance.

DISCUSSION

DISCUSSION

Anemia in the post renal transplant setting is a relatively common feature. This has been a subject of analysis in various transplant centres only in recent times. This is probably because the problem of anemia and its refractoriness to treatment (erythropoietin), despite satisfactory allograft function, is being increasingly appreciated in surviving patients. Number of other factors may contribute to this anemia. Known factors like delayed graft function and hypertension were not common in patients taken up for this study.

The studies quoted by yorgin et al. Tresam found 8.5% severe Anemia, in our study found 35% (<11gms) of patients severely anemic[44].

37.5% patients had serum iron of less than 75 mgs %, NAAC Review showed prevalence of iron deficiency 21.1% in one study, 41% in other study. The iron depletion in the immediate post transplant patient due to dialysis or rapid erythropoietin response could not be considered as a contributing factors. Among 4 female patients 2 had abnormal uterine bleeding with anovulatory cycles. 2 patients had recurrent diarrohea with features suggestive of malabsorption, the others showed

appreciable mild response to oral iron, indicating possible nutritional deficiency.

Among 3 patients (7.5%) presenting with macrocytic anemia. One was a chronic alchoholic in the pre transplant period, two other female patients had co-existing hypothyroidism and were on thyroxine substitution.

The major chunk of our study patients presented with normocytic anemia. In these patients that the possibility of other factor, contributing to the anemia had to be deciphered. The mean post transplant GFR achieved 6 months after transplant in our study was 54.92 ml/m/1.73m^2 indicating that 32.5% were in stage 2 and 67.5% were in stage 3 CKDT. The potential risk of decreasing renal function with time, when compared to non-transplanted individuals, clearly sets these individual at a higher risk of anemia. The focus therefore need to shift towards maintaining long-term graft function. Date from UK renal registry show successful management of anemia in patients with CKD stage 4T & 5T.

72.5% of patients showed an increased erythropoietin level indicating the healthy allograft response, however, this function did not reflect in the hemoglobin level. This indicated that other factors could be

impeding the normal hematopoietic response to erythropoietin. An attempt to identify an underlying chronic inflammatory activity, - persisting pre transplant disease activity, - or post transplant chronic immunoreactivity, - was made while analysing these patients.

Above 90% of patients in the 3rd, 4th decade, presented with chronic glomerular disease and chronic interstitial nephritis as the cause for the CKD. Diseases contributing to CKD in the elderly like DM, SHT contributed to 10% only. Good number of them required concommitant treatment of the precipitating disease process which could also contribute to the altered internal milleu.

The following features supported our suspicion of a persistent chronic inflammatory state contributing to the anemia.

1. As already indicated the GFR of the patient had improved to a functional status of statge 2, 3 CKDT. A commensurate Hb increment however, was not noted. P value - 0.8749.

2. The erythropoietin level showed an increase commensurate with the return of glomerular function. (P value – 0.0059). However, similar improvement in Hb was not observed.

3. The LDH was increased to appreciable level in most of the patients. A hemolytic response probably contributing to this increase was not supported in any of our patients with an appreciable reticulocyte count increase. The LDH increase, therefore probably reflects the persisting kidney disease / inflammatory activity.

4. Total iron binding capacity showed an appreciable increase, higher than what one would expect for a given hemoglobin level. TIBC include ferritin and transferrin both of which can respond as acute phase reactants. The significant increase noted in our study probably reflects the response to underlying chronic disease activity.

5. CRP value in our study was within normal range in all our patients. A more sensitive hs CRP evaluation would have reflected any coexisting inflammatory activity. Therefore serum LDH and TIBC were analysed in this direction (Ref: AbrahamN.CartyR, DufourD, Henry's clinical diagnosis and management by laboratory methods chapt. 20). Other studies evaluating post renal transplant anemia has shown the need for IL6 and hepcidin level to indicate or exclude a chronic inflammatory state.

6. Anemia and inflammation

- ACD is observed in many condition associated with chronic inflammation. This normocytic (or moderately microcytic), normochromic anemia is characterised by low serum iron levels, low-normal TF saturation, and an elevated ferritinemia. In contrast to iron deficiency anemia patients are not responsive to iron supply.

- Both cytokines (such as IL-I, IL-6, LPS and TNFα) and hepcidin are implicated in iron metabolism disturbances characteristic of ACD. During chronic inflammation, erythrophagocytosis rate is increased as a result of macrophage activation and cytokine induced red blood cell membrane alterations, an effect mostly driven by TNFα. This result is reduced RBC lifespan. In addition, iron efflux from macrophages is restricted, as a consequence of various events: downregulation of FPN gene transcription, increased expression of hepcidin, which reduces the amount of FPN present at the cell surface, and finally activation of ferritin gene transcription. All of these effects are mediated by pro-inflammatory cytokines and concur to induce sequestration of iron into macrophages and reduction of

plasma iron or hypoferremia. This diversion of iron from the circulation into storage sites is associated with subsequent limitation of the availability of iron for erythroid progenitor cells and onset of iron-restricted erythropoiesis. The latter effect is moreover amplified by pro-inflammatory cytokines (in particular IL-I and TNFα), which inhibit erythropoietin synthesis and proliferation of erythroid progenitor. (Ref : Recycling iron in Normal and Pathological States – Carole Beaumont and Constance Delaby). (Seminars in Hematology, Vol 46, No.4, October 2009, pp 328-338).

- Therefore more comprehensive analysis of anemia of chronic disease with persisting Inflammation could be made with evaluation of Inflammatory cytokine particularly IL6 and hepcidin.

CONCLUSION

CONCLUSION

The study included 40 patients of post renal transplant anemia (6 month after the renal transplant), and analysis of the nature of anemia evaluated.

- Only 12.5% of patient had Iron deficiency anemia and 3 patients (7.5%) had factors not related to CKD, contributor to the anemia.

- The remaining 80% of the patient had anemia related to the chronic kidney disease.

- The analysis supported the possibility of the etiopathogenic factors persisting in the post transplant period, with chronic inflammatory response.

- The Drugs used predominately in our patients (Cyclosporine, MMF, Azathioprine, Tacrolimus) are usually known to produce myelosuppression. Although this could have contributed to the anemia especially in those patient with low reticlocyte response, concommitant reduction in leucocyte and platelet counts were not noted.

- The concommitant use of anti inflammatory drug including those with targeted activity - anti IL6 (TOCILIZUMAB) could enhance the erythropoietin response in hematopoiesis in a majority of patients.

- With a strong possibility of chronic Inflammatory response contributing to Refractoriness to erythropoietin mediatated hemoglobinzation.

LIMITATIONS

LIMITATIONS

- Only a small number has been included in our study
- CRP is not a sensitive indicator of disease activity
- hs CRP which is the better indicator of disease activity could not be performed in our patients.
- Therefore correlative analysis had to be made with serum LDH and serum TIBC. However a more sensitive indicator would be Serum IL-6 and Hepcidin levels which have not been done.
- Analysis of patients with a higher post transplant period (One year and above, 2 years and above.....) would provide more corroborative evidence in this regard.
- Patients have not been treated with erythropoietin in our study. Therefore, resistance to exogenous EPO therapy could not be evaluated.
- Very limited data on post transplant anemia studies is available, Including studies on Indian patients. Therefore a comparative analysis could not be made in a comprehensive manner.

AREAS OF FUTURE RESEARCH

Sequential evaluation of patients in a chronological framework (6 months post transplant, 1 year post transplant, $1^{1/2}$ years post transplant.....) would help evaluate changes in GFR and also variabilities in factors influencing post transplant anemia as demonstrated in our study and also recorded in other similar studies. The chronic inflammatory activity seems to play an important role in the lack of response to erythropoietin in these patients with anemia. Therefore evaluation of IL6 and hepcidin levels in post transplant patient with refractory anemia would be highly sensitive parameters, which could also be used as justification for inclusion of drugs like anti IL6 in the management strategy.

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BIBLIOGRAPHY

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ANNEXURES

PROFORMA

ANEMIA IN POST RENAL TRANSPLANT PATIENTS

Name : Age : Sex : OP.NO :

Address : Ph.No :

Age at Transplant

Nature kidney disease Dm/SHT/CGN/ADPKD/Others

Comorbid illness

Transplant related information

a. Pre transplant Hb
b. Immunosuppressive drugs
c. Blood loss
d. Graft rejection
e. Other medication

EXAMINATION :

Pallor : Jaundice :

Lymphadenopathy : Cynaosis :

Clubbing : Pedal edema :

Vital Signs : BP- PR- Temp - RR-

CVS :

RS :

Abdomen :

Genitalia :

Per rectal / per vaginal examination (in females) :

INVESTIGATIONS :

CBC

HB% : PCV : ESR : Reticulocyte count :

TC : P : L : E : B : M : Platelets :

Red cell indices : MCV : MCH : MCHC : RDW :

Occult Blood in Stools :

Stool for ova & cyst :

Serum Iron & Feritin

LDH CRP GFR

Serum Erythropoietin

PATIENT CONSENT FORM

Title	:	Anemia in post renal transplant patients
Study centre	:	Govt.general hospital, MMC,chennai
Patient's name	:	
Patient's age	:	
Identification number	:	

I confirm that I have understood the purpose of the procedures of the above study. I have had the opportunity to ask questions and all my questions have been answered satisfactorily. ☐

I understand that my participation in the study is entirely voluntary and that I am free to withdraw from the study at any time without my legal rights being affected ☐

I understand that sponsors of the study, others working on sponsor's behalf, the ethics committee and the regulatory authorities
will not need my permission to look at my health records both in respect of current study and any future research that may be conducted in relation to it; even if I withdraw from the study I agree to this access. However I understand that my identity will not be revealed in any information released to third parties unless required by law. I agree not to restrict the use of any data or results arising from the study. ☐

I agree to take part in the above the study an to comply with the instructions given during the study and inform about any change in my health status to the investigator. ☐

I hereby give permission to undergo complete clinical examination and investigations as part of the study. ☐

Signature of the patient

Patient's name and address place
date

Signature of the investigator

Investigator's name place date

37

INSTITUTIONAL ETHICAL COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-600 003

Telephone 25363970
Fax 044 2535115

L.Dis.No.14597/ME5/Ethics Dean/MMC/2010 Dated : 12.05.2010

Title of the work : "Anemia in Post Renal Transplant Patients".

Principal Investigator : Dr. G. Geetha
Designation : PG in MD General Medicine
Department :
Madras Medical College & GGH, Ch-3.

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 12th May 2010 at 2.p.m in Pharmacology Seminar Hall, Madras Medical College, Chennai -3

The members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
4. You should not deviate form the area of the work for which you applied for ethical clearance.
5. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
6. You should abide to the rules and regulation of the institution(s).
7. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
8. You should submit the summary of the work to the ethical committee on completion of the work.
9. You should not claim funds from the Institution while doing the work or on completion.
10. You should understand that the members of IEC have the right to monitor the work with prior intimation.

SECRETARY
IEC, MMC,CHENNAI

CHAIRMAN
IEC,MMC, CHENNAI

DEAN
MADRAS MEDICAL COLLEGE,
CHENNAI -3

MASTER CHART

NAME	AGE	SEX	KD	COM	pre t Hb	ISD	HB	PCV	MCV	MCH	MCHC	TC	PLT	RC./	PSS	IRON	TIBC	CRP	LDH	GFR	EPO	SR.CR
SUDARRAJ	33	M	CGN	DM	8.5	Y	6	19.6	100	33	34	6400	178,000	1.2	normo normo poikilo	70	543	neg	300	44 ML	71.6	1.78
KAVITHA	28	F	CGN	NIL	8.2	Y	7.9	24	98	31	32	4300	178,000	1	hypo micro poikilo	36.4	965	neg	321	42ML	62.8	2.2
NADIYA	24	F	CGN	NIL	10	Y	7.8	24	87	27	32	5800	214,000	0.5	nn/hypo micro	73	479	neg	480	32ML	51.9	1.9
NIJAMUDEEN	25	M	CGN	NIL	8.5	Y	9	26	85	28	34	10,600	60,000	<0.5	hypo micro poikilo	64.1	335	neg	335	30ML	12.6	2.6
KUMAR	27	M	CIN	SHT	8.2	Y	8.9	27	84	27	32	14,800	249,000	1.6	nn/hypo	52.3	507	neg	318	62ML	120	1.14
BASKARAN	42	M	CIN	NIL	9.6	Y	11	30	82	26	31	12,000	291,000	2	micro hypo	50	485	neg	368	61ML	96	1.1
GANESAN	40	M	CIN	SHT	9	Y	9	30	98	30	30	12,400	278,000	0.8	micro hypo	21.9	753	neg	432	66ML	140	1.2
AMSAR	36	M	DM	SHT	11	Y	8.7	26	85	27	32	3,900	1.39,000	0.5	micro hypo	53.9	328	neg	454	40ML	17.2	2
MURUGANANDAM	37	M	CGN	NIL	11.8	Y	10	32	89	29	32	13,200	225,000	1.2	micro hypo	36.4	598	neg	532	63ML	114	1.3
RAMESH	27	M	CGN	NIL	11	Y	8.7	27	89	28	32	13,200	116,000	1.8	nn/hypo micro	90.3	322	neg	465	30ML	11.9	2.1
VINOTH	42	M	DM	NIL	9.8	Y	6.6	22	87	26	29	6,800	161,000	0.2	micro hypo	18.8	1201	neg	275	61ML	200	1
VELLUSAMY	37	M	CGN	NIL	9.8	Y	7.3	23	100	31	31	3,100	78,000	<0.1	normo normo poikilo	120	1012	neg	324	44ML	4.5	2.8
RAMACHANDRAN	28	M	CGN	NIL	10	Y	8.9	28.5	86	27	31	10,800	192,000	<0.1	normo normo poikilo	64	389	neg	303	56ML	82	1.75
MYTHILI	24	M	OTH	NIL	9.5	Y	6.6	20.4	108	35	32	3,500	264,000	<0.1	nn/macro normo	102	566	neg	423	70ML	159	1
DHANALAKSMI	27	F	CGN	NIL	10	Y	6.8	20	94	31	33	4,100	258,000	0.5	nn/micro hypo	93.3	483	neg	324	70ML	25.6	1.27
VALLI	25	F	CGN	NIL	9.8	Y	6.9	23.4	75	22	29	6,600	252,000	0.5	nn/micro hypo	160	108	neg	324	52ML	54.1	1.2
DHANALAKSMI	30	F	CGN	NIL	10	Y	6.2	20	84	25	30	6,700	138,000	0.5	nn/micro hypo	52.8	572	neg	298	65ML	13.5	1.1
RAMACHANDRAN	41	F	DM	NIL	10.3	Y	2.8	7.9	106	37	35	2,500	15,000	<0.5	micro hypo	150	113	neg	262	43ML	200	1.4
JOTHIPRIYA	23	F	CGN	NIL	11	Y	8	27	105	31	30	6,400	240,000	<0.5	nn/macro normo	78.7	426	neg	295	69ML	102	1
PALANIVEL	40	M	SHT	NIL	9	Y	9.2	26	87	27	32	7,400	213,000	<0.5	normo normo poikilo	78.5	131	neg	324	51ML	60	2.3
MURALITHARAN	37	M	CGN	NIL	10.8	Y	11	33	79	26	33	4,100	1.74,000	0.4	normo normo poikilo	71	817	neg	332	37ML	9.6	2
GOPALAKRISHNAN	33	M	CGN	NIL	9.8	Y	8	26	92	28	30	7,900	1.52,000	<0.5	normo normo poikilo	126	690	neg	275	44ML	11	3
GANESHAN	42	M	DM	NIL	11	Y	10	32.7	95	30	31	11,000	160,000	<0.5	normo normo poikilo	97	960	neg	284	51ML	27.5	1.6

GANAPATHY	37	M	DM	NIL	12	Y	10	32	94	32	33	5,300	227,000	1.2	normo normo poikilo	100	433	neg	234	56ML	66.7	1.4
BALAMURUGAN	27	M	CIN	NIL	11	Y	11	34	84	27	32	7,700	157,000	0.3	micro hypo	23.5	447	neg	524	56ML	196	1.6
RAGINIKANTH	44	M	CIN	SHT	10	Y	8.2	31	85	27	32	6,600	209,000	0.8	nn/micro hypo	138	773	neg	532	41ML	36.7	1.6
RENUKA	27	M	CIN	NIL	9	Y	8	27	74	22	30	4,900	285,000	1.5	micro hypo	42.7	790	neg	623	80ML	21.2	1.1
PRABU	28	M	CGN	NIL	11	Y	9	28	86	28	32	6,500	227,000	<0.5	nn/micro hypo	77	756	neg	542	40ML	61	1.7
SHANTHI	31	F	CIN	NIL	9.8	Y	8	25	87	27	31	8,000	162,000	<0.5	nn/micro hypo	191	475	neg	543	47ML	22.7	1.3
KERISHAL	31	M	DM	NIL	11	Y	7	26	72	19	26	4,600	386,000	1.2	nn/micro hypo	70	646	neg	523	80ML	121	1
VENKATESH	27	M	CGN	NIL	10	Y	11	30	78	24	31	11,500	187,000	0.8	normo normo poikilo	128	983	neg	345	44ML	48	1.2
SIVAKUMAR	44	M	CGN	NIL	9.6	Y	10	30	89	30	33	9,000	2,84.000	1.2	normo normo poikilo	135	968	neg	423	55ML	50	1.6
RAJASEKAR	28	M	CIN	NIL	11	Y	11	31	82	29	35	6,600	172,000	0.6	normo normo poikilo	95	811	neg	523	45ML	45	1.4
PANEERSELVAM	43	M	CGN	NIL	12	Y	11	33	82	28	32	10,900	242,000	0.8	normo normo poikilo	161	953	neg	527	48ML	34	1.2
JUDEANTONY	29	M	CGN	NIL	10	Y	10	32	84	27	32	7,100	166,000	1.6	nn/micro hypo	105	743	neg	612	54ML	42	1.2
PANDI	40	M	DM	NIL	9	Y	11	33	85	28	33	8,600	109,000	1.2	nn/micro hypo	116	905	neg	687	52ML	44	1.2
RAVI	40	M	CGN	NIL	10	Y	9	28	87	30	34	8,900	1.84,000	0.8	nn/micro hypo	111	614	neg	324	83ML	66	1
PARITHA	47	F	DM	NIL	9.8	Y	10.8	33	96	32	33	5,800	187,000	2.1	normo normo poikilo	61.9	648	48	398	87ML	160	1
PARAMASIVAM	21	M	CGN	NIL	10	Y	11	33	87	26	33	8,300	160,000	1.2	normo normo poikilo	163	724	neg	270	55ML	52	1.2
SENTHILKUMAR	37	M	CGN	NIL	9.1	Y	11	34	87	29	33	7,800	1.51,000	1	normo normo poikilo	130	565	neg	251	50ML	61	1.1