INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

University of Alberta

CHRONIC ALLOGRAFT NEPHROPATHY, ULRASTRUCTURAL CHANGES IN PERITUBULAR CAPILLARIES AND THEIR CORRELATION WITH RENAL FUNCTION

BY

Hanan Ahmed Fahmy Abdel-Monem 

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Master of Science

Department of Laboratory Medicine and Pathology

Edmonton, Alberta

Fall 1999

University of Alberta

Library Release Form

Name of Author	:	Hanan Ahmed Fahmy Abdel-Monem
Title of Thesis	:	CHRONIC ALLOGRAFT NEPHROPATHY, ULRASTRUCTURAL CHANGES IN PERITUBULAR CAPILLARIES AND THEIR CORRELATION WITH RENAL FUNCTION
Degree	:	MASTER OF SCIENCE
Year this Degree Granted :		1999

(signed)_____

86 N. Lace Arbor Drive .
The Woodlands,
TX 77382
U.S.A

Dated August 19, 1999

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "CHRONIC ALLOGRAFT NEPHROPATHY: ULTRASTRUCTURAL CHANGES IN PERITUBULAR CAPILLARIES AND THEIR CORRELATION WITH RENAL FUNCTION" submitted by HANAN ABDEL MONEM FAHMY in partial fulfillment of the requirements for the degree of Master of Science

Dr. Kim Solez

Dr. Phil Halloran

Dr. David Rayner

Date : May 19, 1999

Abstract

The purpose of this study was to investigate ultrastructural changes in peritubular capillaries in chronic rejection in comparison to other causes of kidney scaring. This study was conducted on 60 patients who were categorized into 6 groups : baseline group (14 cases), moderate acute rejection group (14 cases) , chronic rejection group in patients treated with cyclosporine (cyclosporine era) (12 cases), pre cyclosporine era chronic rejection group (6 cases), cyclosporine nephrotoxicity group in native kidney group (9 cases), and hypertension group (5 cases).

The correlation between renal function and ultrastructural changes in chronic renal allograft nephropathy was studied. The correlation between the area under the serum creatinine level curve (for periods ranging from 1 week to 3 months) and the Banff score for chronic rejection was tested.Also, the correlation between the Area Under Serum Creatinine Curve and the ultrastructural score was verified. Studying the follow up serum creatinine curve helped to answer the following question : " Is Banff score or ultrastructural score considered predictive of the progression of chronic renal allograft rejection into renal failure?" Five variables were evaluated in the six groups mentioned above: (i) Stereological measurement of peritubular capillary basement membrane thickness. (ii) Capillary basement membrane splitting in terms of fractional proportion of split capillaries.(iii) The severity of splitting, measured as the number of layers of basal lamina material. (iv) Post capillary venule-like transformation was evaluated on the basis of high endothelial transformation and mononuclear cell adherence. In all cases, the

differences among the groups were assessed using the Wilcoxon Rank Sum Test with $\alpha = 0.05$.

This study showed that the increase in the basement membrane thickness, the increase in the percentage of basement membrane splitting and the multilayering (4 or more layers) of basement membrane are characteristic for chronic rejection. Histological evaluation, of protocol biopsies in patients with stable renal allograft function by Banff Schema, showed to be predictive of deterioration of renal allograft function due to chronic allograft nephropathy.

The ultrastructural grading system helps to predict the clinical outcome of the renal biopsies. A score of 7 in the ultrastructural changes is predictive of renal failure due to chronic allograft nephropathy. The ultrastructural score depends on three parameters; number of split capillaries, number of split layers, and the percentage of capillaries circumference showing splitting. This study also showed that there is no correlation between the area under the serum creatinine curve and Banff score for chronic rejection.

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Dr. Kim Solez for his dedication, continuous support, and encouragement. Also, I would like to thank Dr. David Rayner for his valued advice and constructive criticism throughout the whole project. Special thanks to Dr. Philip Halloran for his excellent feedback and input during my work in the thesis.

Finally, I would like to thank the technical staff in the anatomical pathology lab for their assistance and the good job they did preparing the illustrations.

Hanan Fahmy

Table of Contents

Page

List of Tables

Table Page

List of Figures

Chapter(1):

Introduction:

Since the first successful transplant in 1954, human kidney allo-transplantation has been a successful treatment for end stage renal failure. The main complication of transplantation is rejection. Rejection can be classified into hyperacute, acute, and chronic rejection.

Hyperacute rejection develops in the first 72 hours after transplantation. It may begin after starting of the blood flow to the graft. Hyperacute rejection is a type II hypersensitivity reaction. It is due to circulating antibodies against the major blood group antigens or allo-antigens of the donor. The introduction of routine / pre-transplant screening and cross matching techniques has diminished the incidence of this complication (1-4).

Most acute rejection episodes occur in the span of days up to 3 months post-transplant. The frequency of episodes of acute rejection are diminished at the end of the first year l post-transplant. Many acute rejection episodes occurring after the first year are probably due to non-compliance with therapy or other precipitating causes, such as viral infection (5). Pathologically, acute renal rejection can be diagnosed based on the presence of inflammatory infiltrate in interstitium, tubule and vessel wall (6,7) tubulitis is a typical feature of acute rejection (8,9). Tubulitis can be identified pathologically by using semi-qualitative grading. The Banff schema grades tubulitis as mild, moderate, and severe based on the greatest number of infiltrating mononuclear cells in the tubular epithelium per tubular cross section; if tubule is sectioned longitudinally, results are expressed 10 per tubular cells (6,7). It is required that the tubulitis be present in more than one focus in the biopsy (l). Immunohistochemical techniques may be useful in identification of tubulitis , the presence of Leu 7 positive cells is a good marker for the identification of acute rejection(91).

Intimal arteritis is the pathognomic lesion of acute rejection (10,11,12,13,17). The Banff classification distinguishes intimal arteritis, defined as lymphocytic infiltration beneath the endothelium, from transmural arteritis with inflammation in the media and /or with fibrinoid necrosis of the vessel wall. Parenchymal necrosis and /or interstitial hemorrhage were recognized as possible manifestation of severe arteritis. However, because there is the potential for significant sampling error in defining vasculitis, it was agreed in 1997 that the grading should be done on the most severely involved vessel. A designation of severe arteritis now is reserved for those cases with transmural arteritis and /or arterial fibrinoid change and smooth muscle necrosis with accompanying lymphocytic inflammation in the vessel (18). Immunohistochemical studies showed that vascular cell adhesion molecule was increased only in the area with vasculitis or arteritis (14).

In 1997 Banff schema, acute rejection is divided into three types. Type I is tubulointerstitial rejection without arteritis, further divided into type IA with focal

moderate tubulitis and IB with severe tubulitis. Type II vascular rejection, is characterized by intimal arteritis. Further divided into IIA if intimal arteritis mild to moderate, and IIB if severe. Type III, severe rejection is with transmural arteritis with or without fibrinoid and smooth muscle necrosis. Those cases with only mild tubulitis inflammation remain in a "borderline" category (18). The current Banff '97 classification (18) of acute rejection is built on several past schemata(17,19,20,21). Finkelstien et al proposed one of the first semi-quantitative systems. Which grades the interstitial, vascular, and glomerular lesions with resulting acute rejection index (19). Banfi et al had four categories of acute rejection (20). Both these early schemata had more categories at the severest end of spectrum and emphasized thrombotic feature, reflecting the patterns of pathology in that era. Neither recognized endothelialitis as a distinct lesion.

Now an accurate diagnosis of acute rejection can be made by using Banff schema which has shown to be highly reproducible and clinically valid for acute rejection (22). Standardization of renal allograft of renal allograft biopsy interpretation and reporting is necessary to guide therapy in transplant patients and to establish an objective end point for clinical trials of antirejection agents. The Banff schema (6,7) has recently been developed to fill this need and has been clinically validated in numerous studies (23,24,25,26,27).

Rush et al established a protocol in which they biopsied a symptomatic patients at intervals post transplant using Banff criteria. In his study, protocol biopsies revealed a high prevalence of subclinical rejection, as well as, borderline inflammation, despite levels of CsA considered to be in the therapeutic range. Every biopsy was given a score for the severity of the histological changes (the Banff score for inflammatory changes [BSI], which permitted the generation of cumulative BSI over the year of follow-up for each patient. At the end of the year, normal histology and excellent renal function were seen only in transplant patients with the lowest cumulative BSI. These results suggest that repeated inflammation in the renal allograft, even if subclinical, can lead to its assess adequately the effectiveness of current immunosuppressive therapies in renal transplant patients (23).

In 1993, Gaber et al, studied the clinical applicability of the Banff classification for acute rejection. This study demonstrated that SUM score grading the severity of interstitial inflammation, tubulitis, and intimal arteritis were predictive of acute rejection reversal (24).

Recurrent acute rejection remains a significant problem for recipient of renal allografts, with a large proportion of patients progressing to graft loss. In 1996, Gaber et al, used the Banff schema (6,7) to determine whether the histological pattern of acute rejection (severity and scoring) could discriminate recurring from nonrecurring rejections and to examine whether objective rejection scoring had predictive value for rejection reversal and outcome .A total of 67 biopsies obtained from 50 patients with acute rejection were examined for the occurrence of recurrent allograft rejection. All patients were maintained

on cyclosporine -based triple immunosuppressive protocol biopsies and had proven acute rejection without chronic rejection. Rejection recurred in 13 patients (26%), of whom 4 further developed third rejection.

This study demonstrate a significant increase in the score for tubulitis, interstitial inflammation and the total SUM in the third rejection compared with the first rejection. Score for intimal arteritis in successive rejections displayed a similar trend of increasing severity with recurrence of rejection. This increase in morphological severity with multiplicity of rejection has reflected on the response to clinical therapy. Reversal of acute rejection by steroid was achieved in 30%-40% of all treated first and second rejection. recurrent rejection episodes having a significantly higher SUM score were less responsive to anti lymphocyte therapy (25).

A multi-center study, a consortium of 19 transplant center, convened in 1995 to examine kidney transplant rejection. Histological grading of transplants kidney biopsies was done by using Banff schema (6,7). Banff schema biopsy grading was correlated with clinical parameters of rejection and therapy response. This multi-center review of rejection of rejection severity confirms that standardized histological classification such as Banff schema provide a reliable means for stratifying patient risk of treatment success or failure. These data support the use of Banff criteria in clinical trial design (26). Another study suggested that semi-quantitative biopsy reporting systems should be routinely included in clinical databases to allow for prospective examination of structure function relationships and long -term outcomes following acute rejection (27).

Three recent studies have the led to the Banff 97 (18) categorization of acute /active rejection changes as " types " (tubulointerstitial or vascular) rather than " grades " of rejection.

The Roche mycophenolate mofetil study (28) included 87 biopsies scored blinded to clinical history or outcome using the Banff criteria. The highest tubuilits and vasculitis score in the biopsies obtained post-transplant from each case, as defined by Banff 93-95 grading system, were recorded. The finding of vasculitis score of any grade was significantly correlated with allograft loss. Outcome, defined by graft survival, was independent of rejection therapy cohort but highly dependent on type and severity of vascular lesions. Similar finding emerged for the Collaborative clinical trails (17).

In a more recent study, Nickeleit et al analyzed the prognostic significance of vascular lesion in rejection (29).They found that rejection with endarteritis was significantly less responsive to steroid therapy than rejection without endarteritis. One year graft failure was also somewhat higher in group with higher in the group with arteritis (28%) than without (21%), although the difference was not significant. Conversely, severity of interstitial inflammation and tubulitis (defined by CCTT criteria) did not correlate with response to therapy or outcome.

Chronic rejection is the transplanter's next challenge. Chronic rejection remains the principal cause of graft failure after 1 year post-transplantation (30).

The most characteristic feature of chronic rejection, shared by all organs, is concentric generalized arteriosclerosis with low-grade perivasculitis affecting all allograft arteries of the transplant.

The histological criteria of cardiac transplant is atherosclerosis as an expression of chronic rejection have been published by Billingham et al (31) and consist of tubular myointimal hyperplasia in the presence of an intact internal elastic lamina with occasional breaks and with very little, if any, calcification.

The joint proposal from the Alexis Carrel Conference (32) emphasized that the diagnosis of chronic renal rejection should be made on basis of compatible graft histology, such as fibrous intimal thickening and/or transplant glomerulopathy and interstitial fibrosis and tubular atrophy, in conjunction with progressive deterioration in renal function without other cause occurring at least 3 months post-transplantation. The slope of the regression line of reciprocal plasma creatinine over time should be significantly different from zero and should be calculated on the basis of 10 consecutive values of plasma creatinine values over a 3-month interval. Chronic rejection has a temporal component: Deterioration in function occurs over a certain time period. It is proposed to accept a 3-month interval as the minimal observation as the minimal observation period to establish chronicity. It is also proposed to exclude graft loss from chronic rejection in the first 3 months post-transplant since most graft losses in this time period are accounted for by acute or accelerated acute rejection episodes. This does not exclude the possibility that chronic rejection is developing within this early time period (32).

In the Banff schema 93-95 (6,7), it is proposed that if the histological and clinical criteria for chronic kidney graft rejection are met and if a sufficient number of arterial cross sections are present in the biopsy, the rejection should be graded by the severity of the fibrous intimal thickening observed. In Banff schema 97 the grading of chronic interstitial fibrosis and tubular and tubular atrophy and /or loss remains unchanged from Banff schema 93-95 , with quantification based on the percentage of cortical parenchyma involved (18).

The Carrel Conference proposal is consistent with the Banff Schema in that it emphasizes the importance of the vascular and glomerular changes of chronic rejection and proposes to asses the nature and the degree of vascular, glomerular, and tubulo-interstitial involvement to allow evaluation of therapeutic intervention that may act on various compartments (32).

Life table statistics to demonstrate the impact of a new drug regimen on chronic allograft failure require 5 to 10 years to reach significance even with a large number of patients .this period is definitely marginal, if not too long, to develop a new molecule as a drug. Therefore, there is a significant interest in generating new end -points,

intermediate efficacy end-points, which would signify later chronic rejection. Until now, no such end-points have been available (34). But only few studies have documented an association between chronic allograft pathology found in protocol biopsy and subsequent renal allograft function and the role of biopsies in the diagnosis of chronic rejection.

Isoniemi et al found that patients with normal renal function, a 2 -year chronic biopsy score (estimated histologically by chronic allograft damage index) was the single most important predictor for the patients who will proceed to clinical chronic rejection later, and this study suggested that a protocol biopsy (at 2 years or possibly even earlier) (35). Dimeny et al that chronic score correlated with a graft loss and function at 2or3 yr. posttransplant (36). In recent study by Rush, confirmed the association between chronic pathology detected by protocol biopsy and subsequent decline in allograft function at 24 months posttransplant. Moreover, it suggested chronic pathology that was predictive of diminished allograft function may be detected as early as 3 months posttransplant. Also this study confirmed the importance of late clinical rejection and delayed graft function as a risk of elevated serum creatinine and emphasized that the protocol allograft biopsies at 6 months posttransplant could be considered as an early end point to assess the impact of a given immunesuppressive protocol (37) In another recent study, by Seron et al, concluded that early protocol biopsies are useful to detect patients at risk of losing their graft due to chronic transplant nephropathy (38).

Chronic renal allograft rejection is characterized by gradual progression suggesting persistent low-grade injury. A new insight demonstrates the involvement of apoptotic cell death as a potential pathway of chronic rejection. The presence of apoptotic cell death was studied by determining apoptosis-induced oligonucleosomal DNA fragmentation in the biopsy specimen using 3' end labeling with terminal transferase, gel fractionation, and Southern blotting. The specific cell types with increased DNA fragmentation were determined by in situ 3' end labeling performed on sections of the biopsies. Significant DNA fragmentation was found only in the specimens from patients with chronic rejection (81).

There are some studies demonstrated that a variety of cells and molecules may regulate smooth muscle cell replication in the vascular wall, the migration of smooth muscle from the media into the intima, and the development of arteriosclerotic lesions throughout the entire length of the vessel wall. these molecules include peptide growth factors and lipid mediators of inflammation. The role of TGF-beta in pathological processes in the transplanted kidney is beginning to be investigated both in animal models and humans.
In chronic rejection, expression of TGF-beta, message, and induced proteins is elevated, especially in cortex. TGF-beta mRNA, unlike other inflammatory cytokine mRNAs, correlated very well with interstitial fibrosis, a hallmark of chronic rejection. Thus a relationship between renal scarring and TGF-beta has been documented by most studies of transplant kidneys (39).

In the Horvath et al study(40), they examined the altered expression of transforming growth factor-beta isoforms in chronic renal rejection in humans including transforming growth factor beta-1 (TGF-beta 1), TGF-beta 2, TGF-beta 3 and their receptors, transforming growth factor beta receptor type I(T beta R-I) and T beta R-II. using Northern blot analysis and immunohistochemistry. They claimed that there is an increase in TGF-beta 1 and TGF-beta3 immunoreactivity in the glomeruli suggests that there is a redistribution in TGF-beta expression in chronic renal allograft rejection. Together with changes affected by B-cell mediated immunity, the above alterations might contribute to the histopathological changes that occur in this disorder, such as intimal fibrosis, arteriosclerosis and glomerular and tubular sclerosis.

Shihab et al (41) examined the expression of three TGF-beta isoforms (1,2, and 3) and the two matrix proteins induced by TGF-beta that serve as a marker of fibrosis by immunofluorescence technique in acute and chronic rejection of human renal allografts. There was a significant increase in the expression of the three TGF-beta isoforms, fibronectin EDA+ , and plasminogen activator inhibitor -1 (PAI-1) in the tubulointerstitium from all cases of acute rejection and chronic rejection. In the glomeruli of acute rejection cases only TGF-beta1 expression achieved statistical significance, whereas in glomeruli of chronic rejection cases, all the three TGF-beta isoforms in addition to fibronectin EDA+ and PAI-1 were elevated. Because TGF- beta was present both in acute and in chronic rejection and because acute rejection episodes are a good predictor for chronic rejection, these results suggest that TGF-beta may play a role in the pathogenesis of fibrosis in chronic rejection.

Persistent fibrin depositions in kidneys undergoing chronic rejection has been suggested to contribute to the obliteration of the vasculature in these grafts. Fibrinolysis, the process to remove fibrin in tissues, is initiated by tissue type plasminogen activator (tPA) and suppressed by type1 plasminogen activator inhibitor (PAI-1). To investigate their role in chronic rejection and fibrin deposition, Wang et al (42) examined the expression of tPA and PAI-1 in an unmodified chronic rejection model using a Fisher 344 to Lewis rat renal transplant . Their immunohistochemical studies showed PAI-1 was mainly localized to the damaged/proliferative vascular intima. These results suggest that persistent induction of PAI-1 may be responsible for the continuance of fibrin deposition, which is associated with irreversible damage and chronic graft loss. In another study by Shihab et al (43) it has been claimed that the expression mRNA expression of plasminogen activator inhibitor (PAI-1), a protease inhibitor stimulated by TGF-beta 1, increased along with TGF-beta 1 and matrix proteins. These results suggest that the fibrosis of chronic renal allograft rejection
is mediated , at least partly, by the dual action of TGF-beta 1 on matrix deposition and degradation.

The development of angiogenesis and tubulointerstitial lesion during chronic rejection of human renal allograft has been associated with polypeptide mitogen which may serve as an important mediator of growth and repair responses. Kirby, et al (44,45) studied the

immunolocalization of acidic fibroblast growth factor and receptors in the tubulointerstitisal compartment and vascular compartment of chronically rejected human renal allograft. Vascular lesions as well as tubulointerstitial in kidney allografts experiencing chronic rejection demonstrated the exaggerated appearance of FGF-1 ligand and receptors. Immunoreactive FGF-1 readily was detected in medial smooth muscle cells and focal areas intimal hyperplasia, particularly in association with the presence of inflammatory infiltrate. Enhanced staining for FGF-1 mRNA primarily was associated with the appearance of resident inflammatory cells. Medial smooth muscle cells of hyperplastic vascular structures demonstrated the greatest immunoappearance of FGF receptors. However. diffuse immunostaining also was observed in areas of intimal hyperplasia. The enhanced appearance of both FGF-1 and FGF receptors in the vascular wall suggested that this polypeptide mitogen may serve as an important mediator of growth responses associated with neointima development and angiogenesis during chronic rejection of human renal allografts.

Platelet-derived growth factor (PDGF) exists as a dimer composed of two homologous but distinct peptides termed PDGF-A and -B chains, and may exist as AA, AB, and BB isoforms. PDGF-A and -B chains have now been localized at both the mRNA and the protein levels to the intimal proliferative lesions of vascular rejection. These peptides, which are known stimuli for smooth muscle cell migration and proliferation in experimental vascular injury, may have similar stimulatory effects on smooth muscle cells in an autocrine and/or paracrine manner to promote further intimal expansion and lesion progression in this form of human vasculopathy (46).

Pedagogos and et al (47), studied the involvement of myofibroblast (MF) in chronic transplant rejection. MF were a major component of the interstitial infiltrate of 10 patients with chronic transplant rejection. Abnormal persistence of these cells in the interstitium is one of the events that contributes to pathologic scarring of the kidney.

There is some evidence suggesting that lipid abnormalities maybe involved in the pathogenesis of chronic renal allograft rejection (48). There is in vitro and in vivo evidence of increased low density lipoproteins (LDL) oxidation in renal transplant recipients which might facilitate the progression of atherosclerosis and enhance the process of chronic vascular rejection(49).

Chronic rejection is a clinical syndrome characterized by a progressive decline in renal allograft function and nonspecific histologic findings of interstitial fibrosis, glomerulosclerosis, and fibrointimal proliferation of internal arteries. Most late allograft failure that is not due to death with a functioning allograft is caused by chronic rejection. Clinical correlates for chronic renal allograft rejection can be classified into two broad categories: immune (alloantigen-dependent) and non-immune (alloantigen-independent). Non-immune risk factors include donor source (living -related Vs, cadaveric), cold ischemia time, delayed graft function, size mismatching, donor age, donor and recipient gender, recipient race, hyperlipidemia, and hypertension. (50-69) . Clinically, the grafts

most at risk for chronic dysfunction include those from very young donors (donors less than 3 years of age), elderly donors (donors more than 61 years of age), female donors, and African-American donors.

Pediatric kidneys perform badly presumably because of a reduced filtration area associated with their smaller size and inability to tolerate adult levels of renal perfusion. Kidneys from older patients have diminished numbers of nephrons associated with age-dependent sclerosis. The best transplant results were obtained from young donors and the worst were with older donor kidneys regardless of HLA compatibility and up to 21% of kidney failures resulted form insufficient renal mass due to age and were incorrectly attributed to chronic rejection. However, other studies indicate that healthy grandparents provide an excellent population for a living related donation. Kidneys from women placed in men maybe at risk because of the size discrepancy, and kidneys from African-Americans have fewer nephrons than do kidneys from whites, and generally perform less well. These observations have been emphasized by studies in experimental animals (50-69).

Race and ethnicity have an impact on kidney allograft outcome. Asian recipients of renal allograft had the best long-term survival rates. This may be due to the low incidence of sensitization ,the low incidence of acute rejection and chronic rejection leading to graft loss ,and the prevalence of primary disease entities that have been associated with excellent long-term prognoses, especially IgA nephropathy and chronic glomerulonephritis. Hispanic recipients also had excellent short-and long-term graft survival rates. This may be due to having the lowest incidence of early acute rejection episodes compared with all other racial groups, and the limited deleterious effect of ATN on long-term graft survival among Hispanics. The poor overall graft survival for Black recipients may be due to poor HLA matching, a high rate of sensitization and a grim effect of sensitization on graft survival, the high incidences of acute rejection and ATN, and the high incidence of ATN both pre-and post-transplant (50-69).

Functioning nephron mass has been recently implicated as a risk factor for development of chronic rejection of kidney allografts. The data showed that the quantity of functioning renal mass is not only an important independent determinant of the intensity of chronic renal allograft failure, but also a potent modulator of fundamental cellular and molecular components of a complex process. This phenomenon involves antigen-dependent and antigen-independent elements that ultimately result in chronic allograft failure. HLA-DR mismatch has been considered one of the risk factors of chronic rejection. Significant survival benefits maybe achieved by prospective HLA matching if cold ischemia times are limited. The five year graft survival and function was good in patients who received immunoadsorption to remove anti-HLA antibodies (50-69). The impact of prolonged delayed graft function on long-term graft outcome in cadaveric kidney transplantation has been studied. It was concluded that prolonged DGF is associated with a higher incidence of graft failure, particularly secondary to chronic

rejection after 1 year post-transplant. Moreover, acute rejection based on prolonged DGF probably plays a major role in the development of chronic rejection (71).

Yet it is still difficult to distinguish between chronic rejection and other cause of kidney scarring such as denovo or recurrent glomerulo nephritis; cyclosporine nephrotoxicities, diabetic glomerulosclerosis and hypertension (72, 73).

Almost any glomerular lesions can recur or appear as de novo in renal allograft. The incidence of recurrent glomerulonephritis ranged between 1.9% (90,91) and 3.2%.While de novo glomerulonephritis ranged between 1% and 2.1%. (75). Approximately 50% of patients who develop glomerular disease after transplantation eventually lose the graft.

Graft survival from 6 years after transplantation and onward is significantly lower than patients without glomerular disease (76).

Recurrent glomeruler disease is a prominent cause of renal function deterioration and should be separated from rejection related changes (74). Membranoproliferative glomerulonephritis has a high rate of recurrence in allgrafts. Type I has a lower rate of recurrence (30%) when compared with type II (70%), but is a more common cause of end stage renal disease (77) .Focal glomerulosclerosis has an incidence of recurrence approximately 40% and graft failure occurs in 20% of affected patients (78,79). Membranous glomerulonephritis is uncommon complication of renal transplantation, despite the fact that it is the most common cause of idiopathic nephrotic syndrome in adults(80). IgA nephropathy has a recurrence rate of approximately 50%. However it only rarely leads to graft loss (81).

The distinction of de novo from recurrent glomerulonephritis is based mainly on well documented evaluation of the native renal disease of the patient and the donor renal disease before the appearance of glomerulonephritis. There are only three relatively common forms of de novo glomerulonephritis: (1) membranous, (2) acute serum sickness, (3) anti -GBM disease (82,83,84).

Mauer et al reported thickening of glomerular basement membrane, increased mesangial matrix, and arteriolar hyalinosis in most transplanted diabetic patients by 2 years post surgery. Diabetic is caused by systemic effects of the disease, recurrence is simply a matter of time. The benefit of transplantation in these patients resides in the improvement of the quality of life (85).

Chronic cyclosporine nephrotoxicity has been described in solid organ transplantation and immune disease after long -term CyA therapy (86-88). Mihatsch et al describe an obliterative arteriopathy with downstream glomerular sclerosis or collapse in cyclosporine -treated patients. A pattern of striped intersititial fibrosis ensues with a nephron drop out, tubular atrophy and, ultimately, compromised renal function. Renal fibrosis was observe (89). Myers et al have suggested that chronic cyclosporine-induce vasoconstriction causes damage and occlusion of afferent arterioles, leading to

irreversible glomerular and nephron damage by ischemic atrophy. Clinically, chronic cyclosporine nephrotoxicity is characterized by slowly progressive hypertension, azotemia, and proteinuria (86,33). Mihatsch et al (73) emphasized that for differential for differential diagnosis purposes, the fibrous intimal thickening that occurs in the kidney due to atherosclerosis hypertension, diabetes, or as reaction to reduction in renal mass in processes such as pyelonephritis lacks the mononuclear cell infiltrates, foam cells, proliferation of myofibroblasts, and nuclear atypias frequently seen in fibrous intimal thickening of chronic rejection. In these other conditions, the internal elastic lamina is usually preserved and the intimal fibrosis is often eccentric, thus differing from the chronic rejection lesion in which there is frequent disruption of the elastica and concentric intimal thickening.

From the above discussion, we conclude that several common diseases may cause sclerosing changes in all compartment of the kidney and may therefore confused with chronic rejection. Although Mihatsch et al, provide an excellent guide for the distinction between chronic rejection, similar changes brought about by other entities, and cyclosporine induced nephrotoxicity, chronic rejection is not fully characterized yet and the accurate distinction of chronic rejection from other conditions remains challenge for future (91,92).

Because it is often impossible to define the precise cause or causes of chronic allograft damage, the term "chronic/ sclerosing allograft nephropathy" is preferable to" chronic rejection, "which implies allogeneic mechanisms of injury, unless there are specific features to incriminate such a rejection process. However, recognition of those cases that do represent "chronic/recurrent rejection" may be important, as there are preliminary data suggesting that therapy may be efficacious in these cases (93). Biopsies that demonstrate the following lesion : fibrous intimal thickening, chronic transplant glomerulopathy, interstitial fibrosis, are assigned the noncommittal term "chronic transplant nephropathy" as other conditions such as cyclosporine toxicity, hypertension, and reflux can generate these lesions as well (22).

In Banff schema chronic allograft nephropathy is categorized into three grades, grade I (mild), grade II (moderate), grade III (severe).Grade I is defined as mild interstitial fibrosis and tubular atrophy without(a) or with (b) specific changes suggesting chronic rejection. Grade II is defined as moderate interstitial fibrosis and tubular atrophy without (a) or with (b) specific changes suggesting chronic rejection. Grade III is defined as severe interstitial fibrosis and tubular atrophy without (a) or with (b)specific changes suggesting chronic rejection. Glomerular and vascular lesions help define type of chronic allograft nephropathy: chronic/recurrent rejection can be diagnosed if typical vascular lesions are seen. Vascular changes enable identification of kidneys with chronic/sclerosing changes due to chronic rejection. Specific chronic vascular that suggest that vascular changes are due to "chronic rejection" are disruption of the elastica. Proliferation of myofibroblasts in the expanded intima and formation of a second neointima are useful features. Fibrointimal thickening in vessel without these

feature is finding, especially if it is of new onset and is graded, but it is not regarded as specific for chronic rejection. Chronic vascular changes are graded based on the extent of the occlusion of the most severely affected vessels (73,18).

Newly recognized lesions have been described involving the peritubular capillaries of renal allograft (94-98). In 1990 Monga et al demonstrated their original observations of splitting and lamination of peritubular capillary in patient with transplant glomerulopathy (94). In 1992 Monga et al .assessed peritubular capillary changes in 61 renal allograft specimens from the late post-transplant period by electron microscopy (95). Of the specimen examined. 47 (77%) showed peritubular capillary abnormalities. This included all 38 specimens with transplant glomerulopathy, a lesion seen in cases of chronic rejection and characterized by immune -mediated endothelial damage with development of glomerular capillary basement membrane abnormalities present in the same case. Both types of abnormalities frequently accompanied other histologic signs of rejection.

Sequential biopsies showed that changes in the basement membrane abnormalities may allow prediction of later development of transplant glomerulopathy and clinical chronic rejection. The peritubular capillary changes described by Monga et al. occurred at an average of 2 year post-transplant, although severe changes were observed as early as 1 month in one case.

In contrast, Ivanyi et al .(96) studied post-capillary venule-like changes in peritubular capillaries by electron microscopy in 5 cases of acute rejection (biopsies) from 1 to 12 wk post-transplant. Morphometric studies showed a (significant) two-fold increase in endothelial cross sectional area in the acute rejection group compared with others. There was increased adherence and passage of lymphocytes through the endothelium. The greatly enlarged peritubular capillary endothelial cells in acute rejection had increased numbers of mitochondria and ribosomes and were often associated with reduplicated basement membranes. The peritubular capillary basement membrane reduplication observed in both studies may predict later decline in function.

In 1997, G. Lajoie examined the utrastructural changes of peritubular capillaries, over a period of up to 8 months, in 14 biopsy specimens obtained from 5 renal allograft recipients diagnosed with "pure" antibody-mediated rejection. In peritubular capillaries, there is progression of injury from necrosis of endothelial cells with lifting and denudation of basement membrane to complete disappearance of capillaries. Acutely, acute tubular necrosis is a constant finding. At 2 to 3 months post-transplant, the remaining capillaries are dilated, misshapen, and distorted, and are surrounded by reduplicated and thickened basement membrane. These changes are associated with increased interstitial fibrosis and tubular atrophy, comparable to a sort of renal "asphyxial" death. Lajoie concluded that in "pure" antibody-mediated rejection, the endothelium of peritubular capillaries is a main target of injury (97).

Papadimitriou et al. examined 135 biopsy specimens from renal allografts and native kidneys and categorized the ITC basal lamina alterations into 5 patterns. The results showed that although marked ITC basal lamina abnormalities are characteristically seen in association with TG, lesser degrees of these changes may also be found in native kidneys and in transplants with other types of glomerulopathis (98).

The microvasculature of transplanted organs has been recognized to be an early and important target in rejection of various organs (99-101). Given the potential importance of these peritubular capillary lesions in predicting later decline in graft function and in helping to define chronic rejection more accurately (92,94,96) we designed our study. The objectives of this retrospective research are : to study the ultrastructure changes in peritubular capillaries, to measure the basement membrane thickness in chronic allograft rejection, to study the correlation between clinical renal function and ultrastructural changes in chronic renal allograft rejection. This study successfully distinguished between the ultrastructural changes observed in chronic rejection and other ultrastructural changes observed in other causes of kidney scarring.

The presence of the basement membrane thickening in chronic rejection was unique and helped to differentiate between chronic rejection and other causes of kidney scarring. The pattern of splitting (multi-layering) also helped distinguish between chronic rejection and other causes of kidney scarring, especially cyclosporine nephrotoxicity. The study also showed that the multi-layering (4 or more layers) of the basement membrane is specific for chronic rejection. The ultrastructure grading system helped to predict the clinical outcome of the renal biopsies. A score of 7 in ultrastructural changes is predictive of renal failure in chronic allograft rejection . The ultrastructural score depends on three parameters: number of spilt capillaries, number of split layers, and percentage of capillaries' circumferences showing splitting.

Acute rejection is the most important risk factor for chronic rejection (102,103,104). Most studies correlate the number of episodes and the frequency of acute rejection to the occurrence of chronic rejection. Pekka Häyry, et al. studied the intensity and the length of the episodes of acute rejection and how the length and intensity contribute to the development of chronic renal allograft rejection. The study was conducted on experimental animals. The study demonstrated that the area under the serum creatinine curve vs. time (which represents the intensity and the length of episodes of acute rejection) correlates better than the number of acute rejection episodes to the development of chronic rejection (CADI) and to late graft function. Based on the Pekka Häyry, et al. research, this study investigates the correlation between the area under serum creatinine curve vs. time (which represents the intensity and the length of acute rejection episodes from a period of 1 week to 3 months) towards the development of chronic rejection assessed by Banff Schema. This study did not agree with the Pekka Häyry, et al. (105) research. The difference between this study and the Pekka Häyry 's is because Pekka Häyry conducted their study on experimental animals and he had the ability to manipulate the intensity and the length of acute rejection by administrating or

withdrawing the immunosuppressive drugs. To the contrary, our study was a retrospective study conducted on human patients and most of the patients had one or two episodes of acute rejection.

Only a few studies have documented an association between chronic allograft pathology found in protocol biopsy and subsequent renal allograft function and studied the role of protocol biopsies in the diagnosis of chronic rejection (35-37). In our study the study of the follow up serum creatinine curve helped to investigate if histological evaluation of protocol biopsies by Banff schema is predictive of deterioration of renal graft function and graft loss due to chronic allograft nephropathy. Also we studied if the ultrastructural evaluation of protocol biopsies is predictive of deterioration renal function and graft loss due to chronic allograft nephropathy.

This study showed that the histological evaluation of protocol biopsies in patients with stable graft function by using Banff schema (6, 7) was predictive of deterioration of renal allograft function and losing the graft due to chronic allograft nephropathy in five out of six patients.
This result agreed with David rush study (37), Isoniemi study (35), and Seron study, (38) in which they stated the importance of protocol biopsies in predicting the deterioration of renal allograft function and graft loss due to chronic allograft nephropathy. Also at the time of protocol biopsy these patients with stable renal function had the tendency to develop acute rejection, and this observation agreed with seron et al study (38).

There was only one patient who did not show any change in his renal function and remain stable after being diagnosed pathologically as chronic allograft nephropathy by Banff schema (6,7). this patient is consistent with the rest of the group and showed the same degree of severity of pathological changes (CI1 ,CT1 ,CV1) that the rest of the group had . this patient behaved clinically different form the rest of the group for the following reasons: (I) this patient is part of a clinical trial
(mycophenolate mofetil study), and he is well controlled. (II) This patient only have subclinical rejection and this patient may be will develop clinical rejection later.

Also the study of ultrastructural changes in protocol biopsies was predictive of deterioration of graft function and losing the graft due allograft nephropathy the severity of ultrastructural was predictive of graft loss due to chronic allograft nephropathy. As patients having ultrastructural score of 7 or more went to renal failure. In contrast the severity of Banff schema score was not predictive of graft loss due to chronic allograft nephropathy. In this study we concluded that protocol biopsies are useful to detect patients at risk of losing their graft due to chronic transplant nephropathy. Also in this study we emphasized the incorporation of the ultrastructural assessment with histological in the evaluation of protocol biopsies.

Chapter (2)

(II)Method

1.Biopsy Selection:

The study was performed on sixty renal transplant biopsies. These biopsies were categorized into the following six groups:

(a) Baseline Group (1 hr. biopsy) :

This group consists of 14 patients whose biopsies were selected from files at the University of Alberta, Department of Pathology. These biopsies were performed on each patient at the time of transplantations after establishing the vascular anastomosis.

(b) Moderate Acute Rejection :

This group consists of 14 biopsies. These biopsies are selected from the files at the University of Alberta, Department of Pathology. These patients had initially good renal function and responded to anti-rejection therapy (Solumedral, OKT3, ALG or ATG) by showing declining of their creatinine level by 30% after rising to 50% from baseline creatinine. Patients who were dialysis dependent, or had poor renal function, or were oliguric or anuric were excluded. This group consisted of 11 males and 3 females. The biopsies were performed between two weeks and 11 months post transplantation. Ten patients received their transplantation from cadaveric donors and the rest received theirs from living related donors. One patient had two transplantations. Two patients ended up with renal failure and both of them were males.

(c) Chronic Rejection Group :

The biopsies of the patients were performed in the cyclosporine era. This group consists of 12 patients. These biopsies were selected on basis of presence of fibrous intimal thickening (CV-1 /CV-2) according to Banff Schema. Patients with a history of hypertension, diabetes, recurrent or denovo glomerulonephritis are excluded . This group consisted of five males and six females with birth dates ranging between 1942 to 1982. Nine of the patients received their renal transplantation from cadaveric donors and the rest got theirs from living related donors. The donors' ages ranged between 25.52 to 86.16 years. The periods of transplantations were between 3 to 72 months . Based on renal graft function (determined by serum creatinine) for each patient prior to the time of the biopsy , these patients are grouped into two groups.

The first group consists of six protocol biopsies in renal transplants patients with stable graft renal function (see fig 3.8a). the mean serum creatinine for this group was 130±95 μmol/l. These patients had stable renal function over a mean period of 510 days. At the time of the protocol biopsy, these patients (with stable graft function) were evaluated histologically by Banff schema (6,7) and diagnosed as chronic allograft nephropathy. The chronic allograft nephropathy score for these patients ranged from (CI1 ,CT1 , CV1) to (CI2 , CT2 ,CV2).This group of patients at the time of the protocol biopsy had the tendency to have an acute rejection episode (see fig 3.9).

The second group consists of six biopsies of renal transplant patients having progressive deterioration of their renal graft function prior to the of the biopsy.The mean serum creatinine level for this group was 308.5±93 μmol/l. At the time of the biopsy ,this patients were diagnosed as chronic allograft nephropathy by using Banff schema (6,7). The chronic allograft nephropathy score for this group evaluated by Banff schema (6,7) ranged from (CI1,CT1,CV1) to (CI2 ,CT2 ,CV2).

(d) Pre-Cyclosporine Era Chronic Rejection Group :

This group was selected from the files at John Hopkins Hospital. The group consists of six patients. Their biopsies were diagnosed as chronic rejection based on the presence of fibrous intimal thickness (CV1 , CV2). Those patients were neither diabetic nor hypertensive and also did not have a history of recurrent or denovo glomerulonephritis.

(e) Cyclosporine Nephrotoxicity in Native Kidney Group :

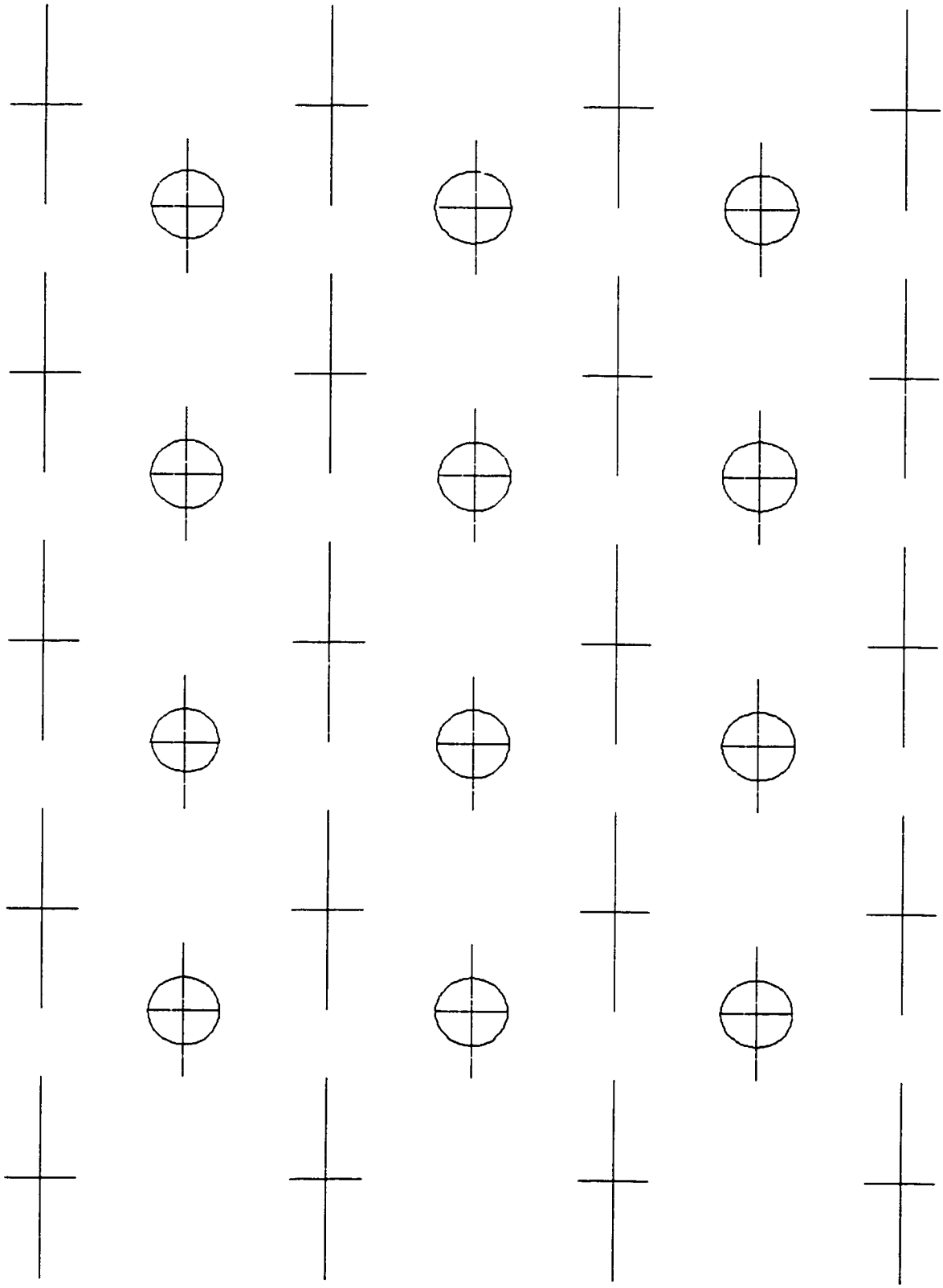
This group of patients was selected from University of Aarhus in Denmark. These patients are treated with cyclosporine for having psoriasis . This group consisted of nine biopsies. These biopsies were diagnosed pathologically as cyclosporine nephrotoxicity due to the sudden onset of hyaline arteriolar thickening described by Mihatch (92). Clinically these group are comparable with the chronic rejection group in this study. At the time of the biopsy the mean serum creatinine for the group was 57.5 ±17.6 μmol/l. The median of serum creatinine for this group was 82 μmol/l.

(f) Hypertension Group :

These patients were selected from files at the University of Alberta Hospital. These patients did not have transplanted kidneys. They showed hypertensive pathological changes in their native kidneys (arterial fibrous initmal thickening). This group consisted of three females and three males. Their ages ranged from 40 to 55 years with blood pressure between 150/100 to 200/110.

(g) Material :

For each case, 15 randomly taken electron microscopic pictures of peritubular capillaries were taken at different levels. Seven pictures were taken at low magnification power (X3000) and eight pictures were taken at high magnification power (X9000).



Figure(2.1)

2- Data Collected :

(a) Measurement of the basement membrane by stereological techniques:

The thickness of peritubular capillary basal lamina is estimated by stereological techniques. Seven EM pictures are randomly taken at different levels and different fields for each case. The thickness is estimated by a coherent test system (grid) that consists of lines and points as shown in figure (2-1).

The thickness is defined by the volume of the barrier divided by one of its two bounding surfaces [Wiebet, 1979, Gunderson, 1984]

$$t = S/v = 2\,(P/I).M.(\Sigma I / \Sigma P)$$

where

S = Surface area of basal lamina.
v = volume of basal lamina.
P = test points falling onto barrier profiles.
I = intersections with the face of reference surface.

The square of the coefficient of error (CE) of the ratio s / v is calculated by Cochran's Formula :

$$CE^2(SV) = \frac{m}{m-1} * \frac{\sum I^2}{(\sum I)^2} + \frac{\sum P^2}{(\sum P)^2} - 2 * \frac{(I * P)}{(\sum I) * (\sum P)}$$

where

m = number of pictures.
P = test points falling onto barrier profiles.
I = intersections with the face of reference surface.

(b) Post Capillary Venule Like Transformation:

a. The thickness of endothelial cell lining of peritubular capillaries is estimated by the same method in which the basement membrane is estimated.

b. Numerical Quantification of Biopsies Showing Post Capillary Venule Like Transformation:

This is estimated by using eight EM pictures of peritubular capillaries at high power magnification (X9000) for each case. This measurement is done by scoring one for each case showing high endothelial cell lining in their peritubular capillaries. A comparison is done between the moderate acute rejection group and control group using Wilcoxon test in which the level of significance, $p < 0.05$.

(c) Basement Membrane Splitting :

The assessment of basement membrane splitting is estimated by two parameters: the percentage of basement membrane splitting and the maximum number of split layers.

(d) Percentage of The Basement Membrane Splitting of Peritubular Capillaries and Their Patterns :

The percentage splitting was estimated by using 8 EM pictures of peritubular capillaries at a magnification power of 9000. A major axis and a minor axis are drawn to divide the capillary circumference into four portions. Then these portions are marked as 0 to 25% , 25% to 50% , 50% to 75% , and 75% to 100%. The capillary circumference for each case is estimated by calculating the average circumference measured in the 8 EM pictures. If more than 60% of the capillary circumference was involved in the splitting, it is considered circumferential splitting. Non circumferential splitting is considered if the measured circumference is less than 60%.

(e) Maximum Number of split Layers in Peritubular Capillaries:

The maximum number of split layers of capillaries for each case is estimated.
The numbers are scored as 1 to 2 layers, 3 to 5 layers, 6 to 8 layers.
A comparison between each group and the control group is performed by using Wilcoxon test in which the level of significance, $p = 0.05$ .This is estimated through 8 EM pictures at a magnification power of 9000.

(f) Numerical Quantification of Split Capillaries:

The number of split capillaries is counted in 8 pictures for each case. If 60% or more of the capillaries is involved in the splitting, it is defined as "diffuse splitting". If less than 60% of the capillaries is involved, it is called "focal splitting". The groups comparison is done by using Wilcoxon test with a level of significance $p<0.05$.

(g) Capillary Profile :

The number of dilated capillaries is counted by using 3 different power fields of light microscopy. A comparison between the chronic rejection group and the control group by using Wilcoxon test.

light microscopy. A comparison between the chronic rejection group and the control group by using Wilcoxon test.

3- Clinical Data Collected :

a. Serum Creatinine Curve :

For each chronic rejection case, serum creatinine level from the time of transplantation to the time of biopsy diagnosed as chronic rejection is plotted using Microsoft Excel.

b. Estimation of Area Under serum creatinine Curve (AUCcr) :

Estimation of the area under the serum creatinine level curve versus time is done for periods ranging from one week to three months. This measurement is also done by performing trapezoidal rule of numerical integration. This method assumes that the area under the curve can be divided into an infinitely small number of trapezoids. The area or each trapezoid is calculated individually and then a summation of these small "elemental" areas is performed to calculate the total area under the curve.

c. Correlation Between the AUCcr and Banff Score for Chronic Rejection :

This was performed by using Spearman's Correlation Biostat Program.
The level of significance p = 0.05

d. Correlation Between the AUCcr and the Ultrastructural Score for chronic rejection :

The ultrastructural score (USCS) is a numerical coding for each biopsy.
A maximum of nine ultrastuctural changes.

Quantitative criteria for membrane splitting score (1 - 3+)

1 - 2 layers -- = 1
3 - 5 layers = 2
6 - 8 layers = 3

Quantitative criteria for capillary circumference score (1 - 3+)

0 - 25% = 1
25% - 60% = 2
60% - 100% = 3

Quantitative criteria for the number of split capillaries score

1 - 3 Capillaries is scored as 1

The correlation is done between the ultrastructural score for chronic rejection (USCS) and the area under serum creatinine curve (AUCcr) by using Spearman's Correlation test with a level of significance p = 0.05 (according to the biostat program).

Follow up Serum Creatinine Curve :
A plot of the serum creatinine versus the time after the biopsy is diagnosed as chronic allograft nephropathy is done by using a Microsoft Excel spread sheet. This is a descriptive study of the renal biopsies function after being diagnosed as chronic allograft nephropathy . The study of the follow up serum creatinine curve helped to investigate if histological evaluation of protocol biopsies by Banff schema is predictive of deterioration of renal graft function and graft loss due to chronic allograft nephropathy . Also we studied if the ultrastructural evaluation of protocol biopsies is predictive of deterioration of renal graft function and graft loss to due renal allograft nephropathy.

4 - Prediction of Biopsy Outcome :

Is the Banff score predictive of the outcome of the biopsy (renal failure or not)?
The failing of chronic renal allograft rejection is scored as 2.
The deterioration in renal function is scored as 1.
The non failing chronic renal allograft rejection is scored as 0.
The pathological score for chronic rejection by Banff schema is plot against the biopsy outcome (failure /non failing). Also, the ultrastructural score for chronic rejection is plot against the outcome of the biopsy by Microsoft Excel.
Statistically, Biostat program was implemented to calculate the mean, median, range, and standard error of range for each group. A comparison is made between the control group and other groups by Wilcoxon test in which the level of significance ,$p < 0.05$.

5 - Tissue Processing :

a. Needle Renal Biopsies:
Renal biopsies are brought to the EM lab. Usually 2 to 3 cores of the tissue are obtained. 20% of each core is used for the EM lab, the rest is for utilization in the histology lab and the immunology lab.

b. Fixation :

b.1. Primary fixation : After cutting the tissue into 1 mm3 portions, they are placed in glutaraldehyde with the temperature kept at 4°C. for 2 hours. After fixation, the specimens are washed in two 15 minutes baths of cocdylate buffer.

b.2. Post Fixation : The tissue is fixed in paladis O504 fixative for 2 hours with the temperature kept at 4°C The specimen is washed for 10 minutes with cocdylate buffer and then for 10 minutes in dH2O.

b.2. Post Fixation : The tissue is fixed in paladis O504 fixative for 2 hours with the temperature kept at 4°C The specimen is washed for 10 minutes with cocdylate buffer and then for 10 minutes in dH2O.

c. Dehydration :

The specimens are dehydrated with graded series of 5 minutes baths of 50%,70%, 80%,and 95% ethanol solutions. These paths are followed by three to 5 minutes in 100% ethanol. The tissue is rinsed in 3 to 5 minute washes of intermediary solution of propylene oxide.

d. Infiltration with Epoxy Resin :

The specimens are introduced to a 50/50 mixture of propylene oxide to infiltrate the tissue for 30 minutes. Then, they are poured off and replaced with epoxy-resin. The vials containing both the tissue and the epoxy-resin are placed on a rotator for a minimum of two hours to ensure complete infiltration.

e. Embedding :

The specimens impregnated with epoxy-resin are embedded by first removing the tissue from the glass vials onto paper. The portions are placed at the bottom of the polyethylene Beem capsules. The Beem capsules are filled with epoxy resin and placed on a labeled tray.

f. Curing :

The embedding specimens are usually placed in a 60°C. oven for 48 hours to cure the epoxy.

g. Cutting :

Blocks are cut by ultramicrotome. Good thin sections that have gold-silver reflections are selected. All sections float on the water's surface which reflects the color of the section in each case. Undesirable sections are removed with an eyelash mounted on a stick.

h. Mounting :

Good gold to silver sections that are 60 nm to 110 nm thick are arranged on water surface and mounted on grids as illustrated. The sections are arranged with an eye lash and touched down by the shiny side of the grid sections are mounted towards the center of the grids.

For each case, two 150 mesh and two 200 mesh grids are prepared. The 150 mesh grids have wider openings and, therefore, more convenient when obtaining low magnification micrographs while the 200 mesh grids allow more support for the sections. The grids are

put in a covered petri dish and placed in an 80 °C oven for at least one hour to allow the sections to adhere properly.

i. Staining :

Grid sections are stained with uranyl acetate and lead citrate.

6-EXAMINATION OF GRIDS USING HITACHI H-600 ELECTRON MICROSCOPE

a- Start up the microscope and ensure the vacuum and the camera are on.

b- Specimen loading : Load the specimen in the specimen rod. Place the specimen rod into the specimen chamber and evacuate the chamber (light goes out) and turn clockwise until the first stop is reached. Continue to turn until the rod is fully inserted.

c- Filament saturation : Ensure that the Lens Systems are in "Zoom" mode. Depress the 50 kV button of the accelerating voltage. Slowly saturate the filament by looking at the HV beam dial (usually saturated at 2 o'clock position).

d- Grid Examination : Place in a suitable objective aperture, and center with objective aperture controls. Manipulate image with vertical and lateral movement controls; magnification controls and brightness controls.

e- Focusing : Lift up the viewing screen to its 45° stop position. Focus your projected image, using the focus fine / coarse and focus under / over controls, and the wobbler if necessary. Lower the viewing screen back to its resting position.

f- Photography : The EM film is a sheet of cellulose acetate which is coated on one side with an emulsion of gelatin containing various halides. Exposure to the electron beam causes the formation of silver nuclei in the silver halide crystals.
- A desired area of peritubular capillaries is selected.
- Adjust the brightness with the controls. Adjust the magnification.
- The film is exposed by the handle on the right of the column ,and by lifting the field screen all the way.
- Take seven different pictures at low magnification (X1000).
-Take the same seven pictures at X 3000 magnification.

g- Development of Negatives : The film is handled only in the dark room setup where a safe light is used to calculate the proper development time by the number of days elapsed.

7 - PRINTING :

a) Developer : Either Dektol or Ilford Multigrade (D-19 Kodak) is used. Fresh developer is prepared as 1 :3 mixture of concentrate to tap water. The working solution is replaced when development time reaches 6 minutes.

b) Stop Bath : Glacial Acetic A., (BDH, reagent grade).

c) Fixer (Kodak) : Ilfospeed Paper Fixer (Ilford). One package of powdered Kodak fixer is added to one Gallon of 25°C tap water.

d) Hypo-eliminator (Kodak) : One package of powdered Kodak cleaning agent is added to one imperial gallons (5 US quarts) of 27 °C tap water.

e) Photo - f10 : 15 ml of Kodak photo f-10 concentrate is added to the tank of ionized water.

f) Setup : The dark room light is turned on for 5 minutes prior to printing. The water is turned on, and run at a temperature of 20°C to 28°C. A gentle flow is all that is required.

g) Procedure :The film is handled only in a dark room setup equipped with a safe light. The proper time for developing the film is calculated by the number of days elapsed since the last development. Three minutes is a typical time for development . The development time usually increases by 15 seconds for each 3 days elapsed since the last development. It may my be increased to obtain a good negative contrast. Remove the negatives from the developer and wash in running tap water for 2 minutes. The negatives are placed in the fixer tank to remove unreduced silver ions and halides .The negative should be left in the solution for 10 minutes . Then the negatives are moved to a second fixer tank for another 10 minutes. Finally, wash the negatives in water for 30 minutes. The washed negatives are dipped into a photo flo solution for 30 seconds and hung up to dry.

h) Exposure : The enlarger lamp is turned on, after placing a negative in the holder. The image is projected onto a piece of white paper and a proper contrast filter is selected for the image. The area on the negative to be printed is selected by moving the paper holder in the proper position. The focus should be checked before undertaking a session. The proper light intensity for exposure is observed and the photometer calibrated accordingly by adjusting the left hand dial on the meter so that the needle on the gauge is set to 50. A sheet of print paper is placed in the paper holder which is set for 8 X 11 inch sheets. The exposure button is pushed and the sheet is exposed for 4 seconds.

Chapter (3)

Results

1. Post-Capillary Venule Like Transformation :

As a first step we studied the post -capillary venule like transformation in peritubular capillaries (i.e the presence of high endothelial cell ,and increase in the adherence and the passage of mononuclear inflammatory cells through the endothelium) in three groups. Biopsies from the moderate acute rejection group [14 out of 14] showed post capillary venule like transformation (fig 3.1). This result was significantly different [p=0.000001] from the control group. Biopsies from chronic rejection showed similar changes to the one seen in acute rejection group, in which 9 out of 11 cases showed post capillary venule like transformation (see fig. 3.2) Chronic rejection group also showed significant difference from the control group (p=0.00007) see table (3.1). Biopsies from acute rejection group and chronic rejection showed post- capillary venule like transformation in their peritubular capillaries. To investigate this further, we measured the endothelial thickness of peritubular capillaries in control, acute, and chronic rejection groups. It was evident that the endothelial cell lining of the moderate acute rejection group was thickened and the stereological estimation shows a mean thickness of 1005 ± 118 nm . This showed that the moderate acute rejection group was statistically significance (with p = 0.03) over the control group displayed in table (3.2) .In the chronic rejection group, the endothelial cell lining of peritubular capillaries was thickened and stereological estimation resulted a mean thickness of 1182 ± 158 nm with statistical significance (p=0.01) over the control group.

2. Basement Membrane Thickness:

We proposed to examine the basement membrane thickness of peritubular capillaries in six groups. The baseline group showed that the mean basement membrane thickness was 504 ± 19.7 nm. In the moderate acute rejection group, the basement membrane was not thickened and the mean thickness is 544 ± 68 nm and showed no statistical significance (p=0.39) over the control group. In contrast , the basement membrane of the chronic rejection group at the cyclosporine era was thickened with a mean thickness of 785 ± 61 nm. This showed strong statistical significance (p = 0.00003) over the control group. It should be mentioned that in pre-cyclosporine era chronic rejection group did not show any peritubular capillaries membrane thickness and the mean thickness is 623 ± 80 nm which showed no statistical significance over the control group (p=0.20). However in both cyclosporine nephrotoxicity and hypertension groups, the basement membrane was not thickened and their mean thicknesses are 593 ±72 nm and 527 ± 60 respectively.

They showed no statistical significance over the control group(p = 0.30 for the nephrotoxicity group and p=0.60 for the hypertension group) see table (3.3).

3. Basement Membrane Splitting

To investigate the changes of the basement membrane of peritubular capillaries further, we propose to quantify the basement splitting. First we measured the percentage of the basement membrane and then we estimated the number of split layers of peritubular capillaries. The study of the basement splitting was conducted on six groups.

3a- Percentage of the basement membrane splitting and their patterns:

Consider the following data provided in table (3.4), moderate acute rejection group shows a noncircumferential splitting of the basement membrane. The mean percentage is 12.7% ± 2.9% which showed statistical significance p=0.0002 over the control group. Figure (3.3). However, biopsies from patients with chronic rejection (at the cyclosporine era) (see fig 3.4) showed changes that were significantly different from the control group and moderate acute rejection group. T here was an increased degree of the basement membrane splitting. The pattern of splitting was circumferential with a mean percentage of 67.2% ± 5.9%. See table (3.4). Similar to the chronic rejection group at the cyclosporine era , the pre-cyclosporine era chronic rejection group showed circumferential splitting of the basement (see fig 3.5) with a mean percentage of 60% ± 1.7%. See table (3.4). This was also statistically significant (p=0.001) over the control group. Similar to the moderate acute rejection and the hypertension group, the cyclosporine nephrotoxicity group showed non-circumferential splitting (see fig 3.6) with a mean percentage of 2.5% ±0.7%, but this showed no statistical significance (p = 0.9%) over the control group. See table (3.4) Refer to figure (3.7), similar to the moderate acute rejection group, the hypertension group showed a non-circumferential splitting of the basement membrane with mean percentage of 23% ± 7.9%. This showed statistical significance (p) of 0.0009 over the control group . See table (3.4).

3.b- Maximum number of split layers in peritubular capillaries:

Refer to table (3.5), the moderate acute rejection group showed an evidence of increase in the number of split layers in peritubular capillaries. See figure(3.3). The maximum number of split layers was 3 and the mean is 2.6 ±0.5. This showed statistical significance (p=0.006) over the control group. However, the chronic rejection group at the cyclosporine era showed more increase in the number of split layers of peritubular capillaries. See fig.(3.4).
The maximum number of layers was 8 and the mean was 5.2 ± 0.6 . This showed strong statistical significance over the control group. Similarly, the pre cyclosporine chronic rejection group showed an increase in the number of split layers. See figure(3.5). The maximum number of layers is 8 with a mean of 4.5 ± 0.8. This showed statistical

significance (p=0.0018) over the control group. In contrast, in the cyclosporine nephrotoxicity on native kidney group, the number of the split layers of basement membrane showed no statistical significance over the control group and the mean no. of split layers is 2.07 ± 1.1 See figure (3.6). The hypertension group showed an increase in the number of split layers of the basement membrane and the maximum no. of split layers was 3 . See table(3.5).

Peritubular Capillary Changes in Chronic Rejection

	Normal	Acute Rejection	Chronic Rejection	CsA Toxicity	Hypertension
Layers	1.7 ± 0.8	2.6 ± 0.5	5.2 ± 1.6	2.7 ± 1.1	2.8 ± 0.4
#> 5	0/12	0/12	5/12	0/12	0/5
#> 4	0/12	0/12	7/12	1/12	0/5
#>3	0/12	0/12	11/12	1/12	0/5
#>2	1/12	12/12	12/12	4/12	4/5

Table (3.6)

To clarify the above data further, a comparison between the number of split layers of basement membrane in chronic renal rejection and other cause of kidney scarring was done. The above table shows that as the number of split layers of peritubular capillary membrane increases, the specificity of the test increases for chronic rejection. On the other hand, with the decrease in the number of split layers of the capillaries basement membrane, the sensitivity of the test increases for chronic rejection increase. The above table shows that the number of split layers of peritubular capillaries (4 or more) is specific for chronic rejection.

4. Numerical quantification of split capillaries:

To clarify the involvement of peritubular capillaries in splitting further, we quantified the number of split capillaries in six group . Refer to table (3.7), the moderate acute rejection group showed an increase in the number of split capillaries. The mean number of split capillaries was 2.7 ± 0.7. This showed statistical significance (p=0.0025) over the control group . In the chronic rejection group at the cyclosporine era, almost all capillaries were split. The mean number of split capillaries was 6.4 ± 0.3 capillaries. This showed strong statistical significance (p=0.000005) over the control group. Similarly, the pre cyclosporine chronic rejection group showed increase in the number of split capillaries was 6.2 ± 0.4. This showed statistical significance (p=0.00012) over the control group .In cyclosporine nephrotoxicity group, fewer capillaries showed splitting, the mean was 1.8 ± 0.5. This showed no statistical significance over the control group. In the hypertension group, there was an increase in the number of split capillaries with a

In the hypertension group, there was an increase in the number of split capillaries with a mean of 5.8 ± 0.5. This demonstrated statistical significance over the control group (p=0.000066).

5. Capillary profile :

To examine the changes of peritubular capillaries further ,the number of dilated peritubular capillaries were quantified by using light microscopy in both chronic rejection group and control group. Refer to table (3.8), the chronic rejection group showed changes in capillary profile in which the capillary became rounded and dilated. The mean number of dilated peritubular capillary is 53 ± 4.5. This showed no statistical significance over the control group.

6. Prediction of the biopsy outcome ;

The study of the follow up serum creatinine curve for each patient, having chronic allograft nephropathy at the time of biopsy, helped to answer the following question: " Is Banff schema or ultrastructural score considered to be predictive of the of the deterioration of renal allograft function and renal failure due chronic allograft nephropathy ?" The chronic allograft nephropathy patients are grouped into two groups based on their renal allograft function prior to the time of the biopsy. The first group consisted of six protocol biopsies for patients having stable renal allograft function (see fig 3.8a). At the time of the biopsy this patients diagnosed as chronic allograft nephropathy by using Banff schema . The mean serum creatinine for this group is 130 ±95 µmol/l .This patients had a stable renal function over a mean period of 519 days. In the follow up serum creatinine curves ,five out six patients showed a progressive deterioration of their renal function and two of them ended with renal failure after 195 days and 305 days. (fig 3.8b ,table 3.9). This study showed that the histological evaluation of protocol biopsies in patients with stable graft function by using Banff schema was predictive of deterioration of renal allograft function and losing the graft due to chronic allograft nephropathy in five out of six patients. There was only one patient who did not show any change in his renal function and remain stable after being diagnosed pathologically as chronic allograft nephropathy by Banff schema . this patient was consistent with the rest of the group and showed the same degree of severity of pathological changes (CI1 ,CT1 ,CV1) that the rest of the rest group had. this patient behaved clinically different form the rest of the group for the following reasons: (I) this patient is part of a clinical trial (mycophenolate mofetil study), and he is well controlled. (II) This patient only have subclinical rejection and this patient may be will develop clinical rejection later.
Also the study of ultrastructural changes in protocol biopsies was predictive of deterioration of graft function and losing the graft due allograft nephropathy .The severity of ultrastructural was predictive of graft loss due to chronic allograft

nephropathy. As patients having ultrastructural score of 7 or more went to renal failure (see fig 3.10 b). In contrast the severity of Banff schema score was not predictive of graft loss due to chronic allograft nephropathy (see fig 3.10 a).

The second group consisted of six patients with progressive increase in their serum creatinine curve prior the time of the biopsy. At the time of the biopsy this patients diagnosed as chronic allograft nephropathy by using Banff schema. The mean serum creatinine level was 308±93 μmol/l. The follow up serum creatinine for this patients showed progressive deterioration of renal function and two out of six patients ended with renal failure. The ultrastructural score for this group of patients was predictive of renal failure due to chronic allograft nephropathy however the severity of Banff schema score was not predictive of graft . loss due to renal allograft nephropathy.

7- Area Under The Curve of Serum Creatinine (AUCcr) and Their correlation With Banff score:

Estimation of the area under serum creatinine curve starts at one week after date of transplant and ends at three months after transplant. This period represents the intensity of acute rejection episodes .In this study there was no correlation between the area under serum creatinine curve and the development of chronic rejection estimated pathologically by Banff schema (by using spearman's correlation $r = -0.2$, $p = 0.4$). Also there was no correlation between the area under serum creatinine curve and the ultrastructural score for chronic rejection ($r = 0.3$, $p = 0.4$).The measurement of the area under serum creatinine is recorded in table (3.9).

Conclusion:

Biopsies from patients with chronic rejection (at cyclosporine and precyclosporine era) show characteristic changes that differed significantly from other groups. See table (3.10). These two groups shows; an increased degree of capillary basement membrane circumferential splitting; increased severity of basement membrane splitting with 4 : 8 layers of basement lamina in comparison with 1 : 3 layers for the control, acute rejection, hypertension, and cyclosporine nephrotoxicity group. Also, there is an increase in proportion of capillary involved in splitting; the chronic rejection group at the cyclosporine era shows a significant basement membrane thickening over the control, acute rejection, hypertension, and cyclosporine nephrotoxicity groups.

Capillary ultrastructural changes	Control Group	Moderate acute rej. group	Chronic rej. at cyclospor. era	Pre cyclos por. era chronic rej. group	Cyclosporine nephro-toxicity	Hyper-tension group
PVC/like transformation	0/14	14/14	9/14			
Endothelial Thickness	698±42 nm	1005 ± 118 nm	182 ± nm			
Basement memb. thickness	504 ±19.7 (nm)	554 ± 68 nm	785 ± 61 nm	623 ± 80 nm	593 ± 80 nm	520 ± 40 nm
% of split capillaries	3.1%±1.1	12.7% ± 2.9	67.2% ± 5.9	60% ± 1.7	2.5% ± 0.7	23% ± 7.9nm
Circumferen-cial	Non-circumf.	Non-circumf.	Circumferen-cial	Circumferen-cial	Non-circumf.	Non-circumf.
Max. no. of split layers per case	1 : 3 layers (17±0.2)	1 : 3 layers (2.6±0.5)	4 : 8 layers (5.2±0.6)	4 : 8 layers (4.5 ± 0.8)	2.07 ± 1.1	2.8 ± 0.4
Proportion of split capillaries	Focal (0.9±0.1)	Focal (2.7± 0.7)	Diffuse (6.4 ± 0.3)	Diffuse (6.2 ± 0.4)	Focal (1.8 ± 0.5)	Diffuse (5.8 ± 0.5)

Table(3.10)

Chapter (4)

Discussion

Chronic rejection is the most important cause of graft failure after 1 year post transplantation (30). Chronic rejection is defined clinically as a gradual deterioration of renal function based on the morphological characteristic of graft biopsy. This definition was proposed in 1992 on the fourth Alexis Carrol Conference on Chronic Rejection and Accelerated Arteriosclerosis (40). Pathologically, chronic rejection is defined by the presence of interstitial fibrosis, tubular atrophy and fibrous intimal thickening.
For pathological description and pathological quantification of the lesion, he Banff Schema (7) should be used.

Chronic cyclosporine nephrotoxicity has been described in solid organ transplantation and immune disease patients after long term cyA therapy (86 -88). The pathology of chronic cyclosporine nephrotoxicity is characterized by tubular atrophy striped interstitial fibrosis and sudden onset of arteriolar hyalinosis (89). It is difficult to distinguish between chronic rejection and other causes of kidney scarring such as cyclosporine nephrotoxicity, diabetic glomerulosclerosis, hypertension infection, recurrent and denovo glomerulonephritis (90 ,91). The accurate distinction of chronic rejection from other condition remains a challenge (91).

From this retrospective study, important ultrastructural peritubular capillaries changes have been identified. These changes help to discriminate between chronic rejection and other lesion causing kidney scarring, e.g. cyclosporine nephrotoxicity and hypertension.

Ultrastructural studies showed significant changes in the morphology of the endothelial cell lining of peritubular capillaries of moderate acute rejection and acute rejection superimposed on chronic rejection groups.

The peritubular capillaries in both moderate acute rejection and acute rejection superimposed on chronic rejection groups showed the presence of high endothelial cell(instead of being squamous) and showed an increase in the adherence and in the passage of mononuclear inflammatory cells through the endothelium. The peritubular capillaries exhibit the morphology of post capillary venule like transformation.
These changes are due to the cytotoxic injury of endothelial cells (96).

Stereological studies showed significant increase in endothelial thickness of peritubular capillary in both moderate acute rejection and chronic rejection (in cyclosporine era) groups. The presence of increased endothelial thickness in chronic group is due to the presence of acute rejection superimposed on chronic rejection. Further more the moderate acute rejection group showed increased degree of capillary basement membrane splitting (up to three layers)/ However there was no significant basement membrane thickness in the acute rejection group.

Based on the above discussion, our study agreed with Ivanyi et al (96) in the presence of post capillary venule like transformation and reduplication of basement membrane of peritubular capillaries in moderate acute rejection. However, in our study the patterns of

Based on the above discussion, our study agreed with Ivanyi et al (96) in the presence of post capillary venule like transformation and reduplication of basement membrane of peritubular capillaries in moderate acute rejection. However, in our study the patterns of basement membrane splitting of peritubular capillaries in the moderate acute rejection group was focal noncircumferential (12.7% of capillary circumference). Moreover, we demonstrated the significant thickness of the endothelial cell lining of peritubular capillaries by implementing stereological techniques.

Biopsies from patients with chronic rejection (during cyclosporine era) showed changes that were significantly different from the control group and the moderate acute rejection group. There was an increase in severity of the basement membrane splitting (4-8 layers). There was an increased degree of capillary basement membrane splitting (circumferential splitting 67% ±1.7) and there was a significant basement membrane thickness. Although the changes seen in precyclosporine era chronic rejection group and chronic rejection during cyclosporine era group were similar, the basement membrane was not thickened in the precylcosporine era chronic rejection group. An important question should be asked regarding the difference of the basement thickness between chronic rejection during cyclosporine era and precyclosporine era chronic rejection - does cyclosporine nephrotoxicity has an impact on the ultrastructural changes of peritubular capillaries?

In corporation of renal allografts from native kidney biopsies from cyclosporine treated patients is important to finely discriminate chronic rejection from cyclosporine nephrotoxicity. In chronic cyclosporine nephrotoxicity on native kidney, the basement membrane of peritubular capillaries was not thickened and showed non-significant, focal, non-circumferential splitting.

It was imperative to discriminate between ultrastructural changes seen in chronic rejection and the ultrastructural changes seen in other causes of kidney scarring. Renal biopsies from hypertensive patients showed significant diffuse noncircumferencial splitting of peritubular capillaries (23%).The maximum number of split layers was three.

Ultrastructural changes of peritubular capillaries showing lamination and splitting are seen in all causes of kidney scarring and moderate acute rejection.However, the pattern of splitting and lamination, the percentage of split of capillary circumference and the number of layers are the main distinguishing factors between all the causes of kidney scarring and chronic rejection. This study showed that the presence of splitting (4 layers or more)of peritubular capillaries is specific of chronic rejection.

In the moderate acute rejection group, the basement membrane of the peritubular capillaries showed significant non-circumferential reduplication. However, the basement membrane of peritubular capillaries in the chronic rejection group showed circumferential splitting(more than 4 layers).

From the pattern of splitting of peritubular capillaries seen in both acute and chronic rejection, it seems that the lesion of the moderate acute rejection may be antecedent of the of the chronic rejection lesion if the moderate acute rejection lesion is repeated. This argument can be supported by the notion that acute rejection is the most important risk

factor of chronic rejection ,and several studies not only have demonstrated the detrimental effect of early acute and especially repeated rejections on the long -term graft survival ,but they also able to show a relation between acute rejection and the subsequent development of biopsy-proven chronic rejection. (86 - 88).

Endothelial cells are not merely targets during rejection episodes; they actively participate through their multiple functions in the secondary inflammatory response (106). Damage to the endothelium of the allograft microvasculature and accumulation of lymphocytes of in the peritubular capillaries and venules are seen in the early stages of rejection (107, 108). The splitting and lamination of peritubular capillaries observed in both acute and chronic rejection maybe due to subsequent repetitive damage of the endothelium which leads to abnormal deposition of excess basal lamina material (109).

Since acute rejection is the most important single factor for chronic rejection (102 - 104), chronic rejection can be predicted by the area under the serum creatinine versus time curve(105). Acute rejection is the most common cause of graft dysfunction in both early and late periods. Our study showed that the measurement of the area under serum creatinine versus time curve(from the period of a week to 3 months which represents the intensity and the length of acute rejection) doesn't predict the occurrence of chronic rejection. Our study did not agree with the Pekka, Häyry, et al. (105) research. The Pekka, Häyry study stated that there is a correlation between the area under serum creatinine Vs. time curve (representing the intensity and length f acute rejection) and chronic rejection. The difference between our study and the Pekka, Häyry 's is because Pekka, Häyry conducted their study on experimental animals and he had the ability to manipulate the intensity and the length of acute rejection by administrating or withdrawing the immunosuppressive drugs. To the contrary, our study was a retrospective study conducted on human patients and most of the patients had one or two episodes of acute rejection.

This study showed that the histological evaluation of protocol biopsies in patients with stable graft function by using Banff schema was predictive of deterioration of renal allograft function and losing the graft due to chronic allograft nephropathy in five out of six patients. This result agreed with David rush study (37), Isoniemi study (35), and Seron study, (38) in which they stated the importance of protocol biopsies in predicting the deterioration of renal function and graft loss due to chronic allograft nephropathy .At the time of protocol biopsy the patients with stable renal function have the tendency to develop acute rejection, and this observation agreed with seron et al study (38). There was only one patient who did not show any change in his renal function and remain stable after being diagnosed pathologically as chronic allograft nephropathy by Banff schema. this patient was consistent with the rest of the group and showed the same degree of severity of pathological changes (CI1 ,CT1 ,CV1) that the rest of the rest group had . this patient behaved clinically different form the rest of the group for the following reasons : (I) this patient is part of a clinical trial (mycophenolate mofetil study), and he is well controlled. (II) This patient only have subclinical rejection and this patient may be will develop clinical rejection later. Also the study of ultrastructural

changes in protocol biopsies was predictive of deterioration of graft function and losing the graft due chronic allograft nephropathy. the severity of ultrastructural was predictive of graft loss due to chronic allograft nephropathy. As patients having ultrastructural score of 7 or more went to renal failure. In contrast the severity of Banff schema score was not predictive of graft loss due to chronic allograft nephropathy. In this study we concluded that protocol biopsies are useful to detect patients at risk of losing their graft due to chronic transplant nephropathy. Also in this study we emphasized the incorporation of the ultrastructural assessment with histological in the evaluation of protocol biopsies.

In conclusion, this study agreed with Monga et al.(94) in the demonstration of splitting and lamination of peritubular capillaries in chronic renal rejection. This study demonstrated that lesion of peritubular capillaries is unique, specific, and predictive of chronic rejection. The increase in basement membrane thickness of peritubular capillaries measured by stereological techniques was unique for chronic rejection and helped to differentiate between chronic rejection and other causes of kidney scarring. This study emphasized on the pattern of splitting and lamination of peritubular capillaries which played a crucial role in finally discriminating between chronic rejection and other causes of kidney scarring especially cyclosporine nephrotoxicity. This study showed that the number of split layers of peritubular capillaries (4 or more split layers) is specific for chronic rejection. This study demonstrated that the severity of ultrastructural changes of peritubular capillaries (ultrastructural score) is not only predictive of deterioration of renal function of the graft, but also is predictive of graft loss.

List of Tables

1) Post-Capillary Venule-like Transformation:

Studying of post-capillary venule like transformation revealed the following results :

REC #	CONTROL	ACUTE	CHRONIC1
1	0	1	1
2	0	1	1
3	0	1	1
4	0	1	1
5	0	1	1
6	0	1	1
7	0	1	1
8	0	1	1
9	0	1	1
10	0	1	1
11	0	1	0
12	0	1	0
13	1	1	0
14	0	1	0

Table (3.1)

Table (3.1) demonstrates the number of cases showing post capillary venule like transformation in control group ,acute rejection group , and chronic rejection Group.

Control = Control group
Acute = Acute rejection group
Chronic1 = Chronic rejection at cyclosporine era group.

Study of Endothelial Cell Thickness:

REC.#	CONTROL	ACUTE	CHRONIC1
1	790	725	1557
2	497	1055	630
3	563	510	1412
4	833	593	1231
5	633	843	592
6	960	639	1851
7	666	833	1068
8	977	913	543
9	769	1372	1041
10	744	1225	920
11	787	1544	2166
Mean Thickness	698 ± 166 nm	1005 ± 118 nm	1182 ± 158 nm
Median	743	843	1068
Wilcoxon Test		p = 0.03	p = 0.01

Table (3.2)

CONTROL = Control group.
ACUTE = Acute rejection group.
CHRONIC1 = Chronic rejection at cyclosporine era group.

Table 3.2 represents the estimation of endothelial thickness of peritubular capillaries in chronic rejection , moderate acute rejection , and control groups.

2) Basement Membrane Thickness:

REC. #	Control.	Acute.	Chronic1.	Chronic2.	Cyclosporine	Hypertension
1	468.75	416.66	700	748	677	520
2	572.916	310.475	897	740	416	518
3	502.136	721.11	727.63	706	463	480
4	416.67	458.333	846.78	395	750	550
5	467.17	652.1739	793.651	833	474	570
6	431.00	493.00	551.80	373	659	
7	598.00	1151.90	639.88		617	
8	534.00	1250.00	705		451	
9	530.03	406.00	1381		833	
10	416.00	575.00	1000			
11	606.061	648.148	876.45			
12	625.00	763.88	798.20			
13	465.00	488.95				
14	632.00	464.286				
Mean Thickness	504 ± 19.7	544 ± 68	785 ± 61	623 ± 80	593 ± 72	527 ± 60
Median	502.13	533.13	795.90			520
Range	216.000	939.50	829.20			
Wilcoxon Test		p = 0.39	p = 0.00003	p = 0.20	p = 0.30	p = 0.60

Table (3.3)

T able (3.3) shows the estimation of the basement membrane thickness in following groups :

Acute = Acute Rejection group.
Chronic1 = Chronic rejection at cyclosporine era group.
Chronic2 = Chronic rejection group before cyclosporine era group.
Cyclosporine= Cyclosporine nephrotoxicity group.
Hypertension= Hypertension group.

3) Basement Membrane Splitting:

3a- Percentage of the basement membrane splitting and their patterns:

REC.#	Control	Acute	Chronic1	Chronic2	Cyclosporine	Hypertension
1	1	13	52	90	2	18.5
2	0	30	82	100	0	20
3	0	1.3	100	71	5	16.5
4	10	28	81	5.7	1	23.7
5	3.2	10	79	85	2	36.5
6	0.5	1.3	80	10	0	
7	10	22	40		0	
8	4	38	30		5	
9	9	7	60		7	
10	0	10.5	70		1	
11	0	11	81		7	
12	0	1.3	52		3	
Mean	3.3 ± 1.07	12.7 ± 2.9	67.2 ± 5.9	60 ± 17	2.5 ± 0.7	23 ± 7.9
Median	1.0	10	74	78	2	20
Range	10	36.7	70	94	7	20
Wilcoxon Test		p = 0.002	p = 0.00001	p = 0.0019	P = 0.9	P = 0.0009

Table (3.4)

Table (3.4) shows *Percentage of split circumference of peritubular capillaries in following groups :*

Acute = moderate acute rejection group.

Chronic1 = cyclosporine era chronic rejection group .

Chronic2 = precyclosporine era chronic rejection group.

Cyclosporine = cyclosporine nephrotoxicity group.

Hypertension = hypertention group.

3.b- Maximum number of split layers in peritubular capillaries:

REC #	Control	Acute	Chronic1	Chronic2	Cyclosporine	Hypertension
1	3	3	5	4	3	3
2	1	3	8	5	3	3
3	2	3	4	8	1	2
4	1	3	3	3	1	3
5	1	3	5	5	5	3
6	1	3	7	2	1	
7	1	3	4		1	
8	2	3	5		2	
9	1	3	8		2	
10	2	3	4		2	
11	2	3	4		2	
12	2	3	5		3	
Mean	1.7±0.8	2.6±0.5	5.2±1.6	4.5±0.8	2.07±1.1	2.8±0.4
Median	2	3	5	4.5	2	3
Wilcoxon		0.006	0.0000049	0.0018	0.3	0.01

Table (3.5)

Table (3.5) shows Number of split layers of peritubular capillaries in the following groups:

Control = Control group

Acute = Moderate acute rejection group

Chronic = Chronic rejection group in cyclosporine era

Chronic2 = Chronic rejection before cyclosporine era

Cyclosporine == Cyclosporine group

Hypertension = Hypertension group

4.- Numerical quantification of split capillaries:

Rec. #1	Control.	Acute	Chronic1	Chronic2	Cyclosporine	Hypertension
1	1	1	4	7	3	5
2	0	3	7	7	7	7
3	0	1	7	7	0	4
4	1	1	7	5	0	7
5	1	1	7	7	2	6
6	2	4	7	7	1	
7	2	5	7	4	1	
8	1	5	5		0	
9	1	2	5		1	
10	1	6	7		3	
11	0	1	7		3	
12	1	3	7		2	
Mean	0.9 ± 0.1	2.7 ± 0.4	6.4 ± 0.3	6.2 ± 0.2	1.8 ± 0.5	5.8 ± 0.5
Median	1	3	7	7	1	6
Wilcoxon		p=0.0025	p=0.0000055	p=0.0001	p=0.01	p=0.0006

Table (3.7)

Table (3.7) shows the *Number of split capillaries in the following groups :*

Control = Control group

Acute = Moderate acute rejection group

Chronic1 = Chronic rejection in cyclosporine era group

Chronic2 = Pre cyclosporine era chronic rejection group

Cyclosporine = Cyclosporine nephrotoxicity group

Hypertension = Hypertension group

5) Capillary profile:

Rec #	Dil. ch.	Dil. b.
1	45	8
2	55	0
3	33	33
4	48	50
5	66	73
6	43	40
7	79	30
8	84	55
9	41	38
10	48	49
11	42	
12	55	

Table (3.8)

Dil. Ch. =number of dilated capillaries in the chronic rejection group.
Dil. b. = number of dilated capillaries in the baseline group.

Case No	AUCcr	BANFS	USCS	TX P Months	BX DATE	CR L @ BX uMOL/L	BX OUTCOME	DATE OF FAILURE	FAIL PERIOD DAYS	LAST CR LEVEL uMOL/L	CREATININE PATTERN	
											BEFORE BX.	AFTER BX.
2	26502 20	6 00	8 00	27 00	20-Sep-94	407 00	2	03-Apr-95	195	629 00	Normal fluctuation with slight increase	Progressive increase with renal failure
5	8287 50	8 00	9 00	72 00	22-Sep-94	346 00	2	05-Feb-96	501	590 00	Progressive increase	Progressive increase with renal failure
6	24521 10	4 00	8 00	3 00	19-Jun-92	915 00	2	20-Apr-93	305	802 00	Normal fluctuation with slight increase	Progressive increase with renal failure
10	5556 50	6 00	9 00	60 00	30-Sep-92	152 00	2	28-Feb-94	516	547 00	Progressive increase	Progressive increase with renal failure
9	12528 20	6 00	6 00	72 00	20-May-94	326 00	1	NO FAILURE	N	592 00	Progressive increase	Progressive increase
7	8841 50	3 00	4 00	37 00	12-Jun-93	206 00	1	NO FAILURE	N	242 00	Progressive increase	Progressive increase
4	9125 50	5 00	6 00	72 00	04-Feb-92	196 00	0	NO FAILURE	N	214 00	Sudden rise	Drop - stable for 2 years, then progressive increase
3	21900 00	3 00	6 00	12 00	06-Jun-94	172 00	0	NO FAILURE	N	127 00	Normal fluctuation	Gradual decrease
8	5609 00	5 00	7 00	7 00	30-Dec-94	168 00	1	NO FAILURE	N	140 00	Several peaks	Progressive increase
1	24838 00	4 00	4 00	9 00	17-Jun-94	112 00	0	NO FAILURE	N	117 00	Normal fluctuation	Gradual drop

Table (3.9)
Clinical Data of Chronic Rejection Patients

0 = no change in renal function
1 = deterioration in renal function
2 = renal failure

AUCcr = Area Under the Serum Creatinine Curve
BANFS = Banff Score for Chronic Rejection
USCS = Ultrastructural Score of Peritubular Capillaries in Chronic Rejection
CRL @ Bx = Creatinine Level at the Biopsy Date

List of Figures

Fig. (3.1)

Electron microscopic picture of moderate acute rejection (9000X) , showing post capillary venule like transformation (high endothelial cell), non-circumferential splitting of the basement membrane into 2 layers, and passage of mononuclear inflammatory cell in between.

Fig. (3.2)

Chronic rejection, showing post capillary venule like transformation, non circumferential splitting of the basement membrane with a maximum of 6 layers partially seen at the bottom right. The electron microscopic amplification factor is 9000X.

Fig. (3.3)

Moderate acute rejection , showing noncircumferential splitting of the basement membrane. The basement split in2 layers seen at the left side

Fig.(3.4)
Chronic rejection, showing circumferential splitting of the basement membrane. with basement membrane split into 5 layers. Electron microscopic picture X 9000.

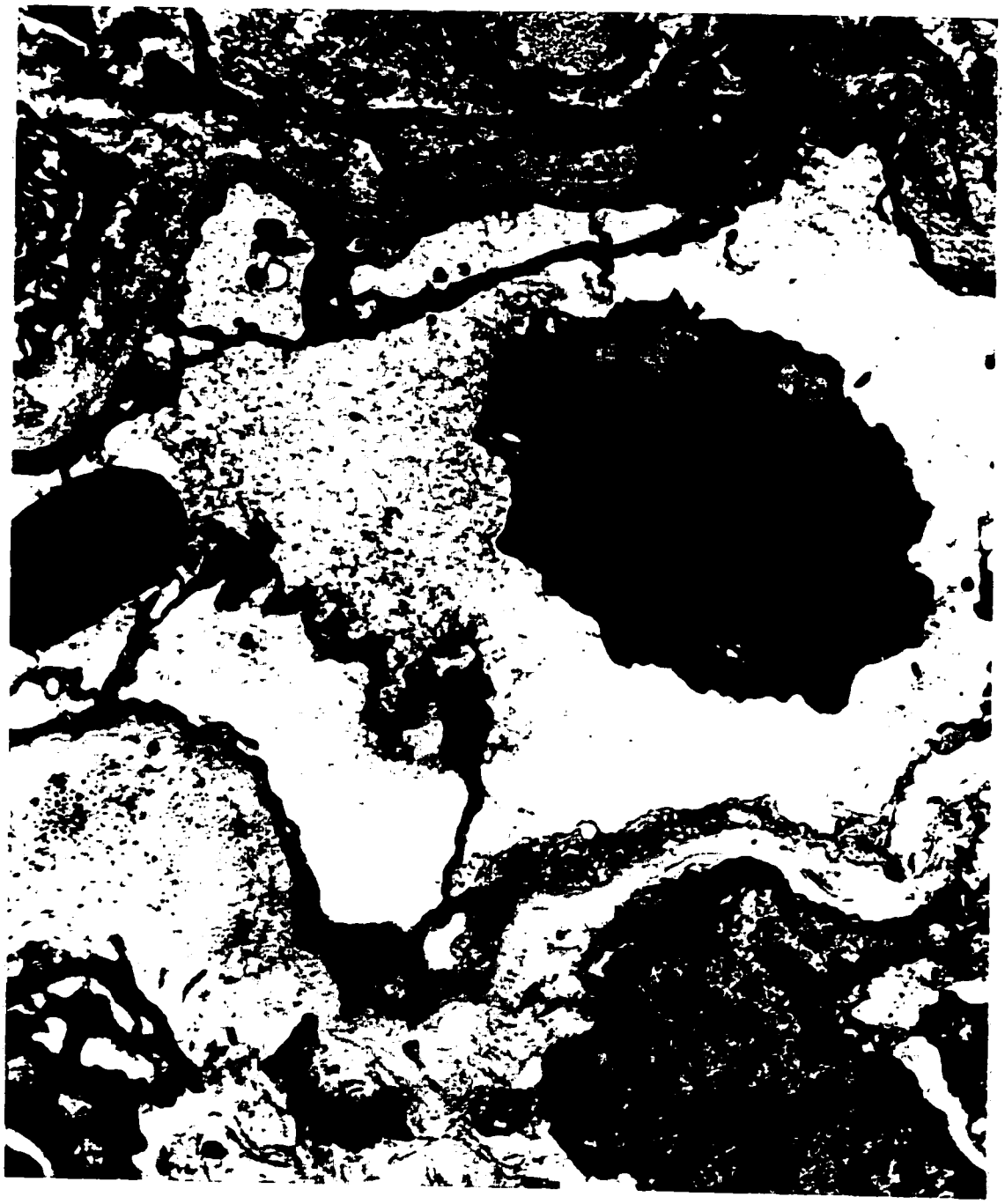


Fig. (3.5-a)

Fig.(3.5-b)

Precyclosporine era chronic rejection, showing circumferential splitting of the basement membrane. The layers are closely packed together. The basement membrane split into 5 layers particularly seen at the top center of fig.(3.5-b).Electron microscopic picture X 9000.

Fig.(3.6)

Cyclosporine nephrotoxicity on native kidney, showing noncircumferential splitting of the basement membrane. This is particularly seen at the arrows. The basement membrane split into 2 layers.

Electron Microscopic picture 9000 X

Fig.(3.7)

Hypertension, showing noncircumferential splitting of the basement membrane, particularly seen at the arrows of the picture. EM 9000 X

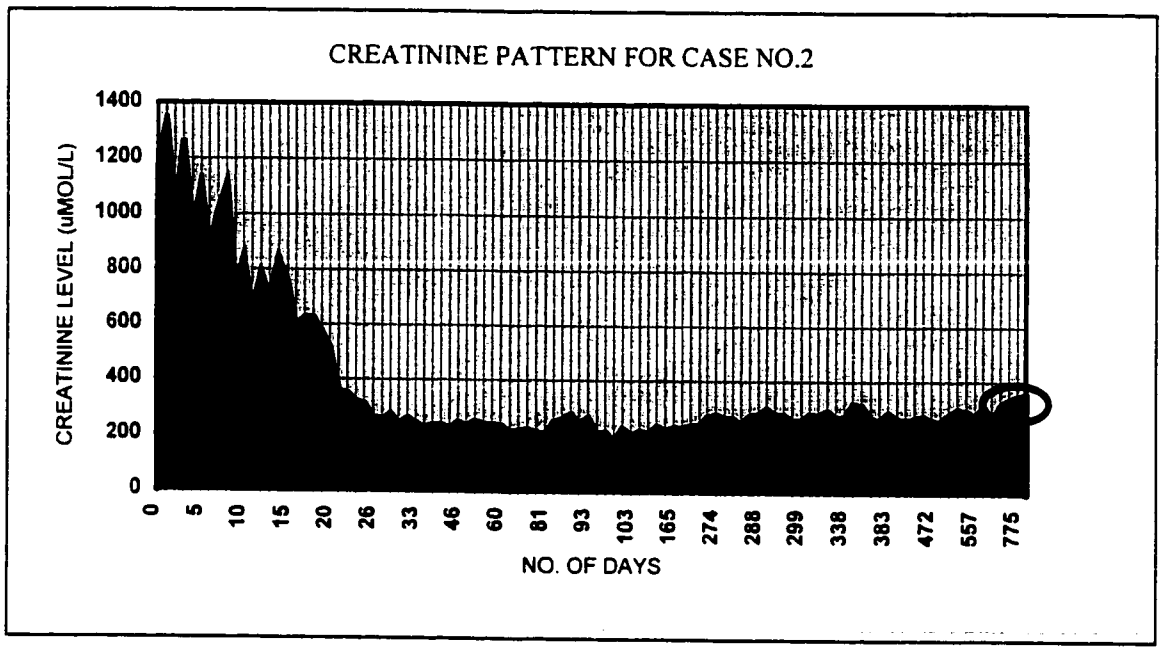


Fig.(3.8a)

This graph represents serum creatinine level for patient with stable renal function. Figure (3.8a) shows a slight increase in pattern the serum creatinine level prior to the time of protocol biopsy . At the time of the protocol biopsy this patient diagnosed as chronic transplant nephropathy .

The circle represent the time of performing the protocol biopsy

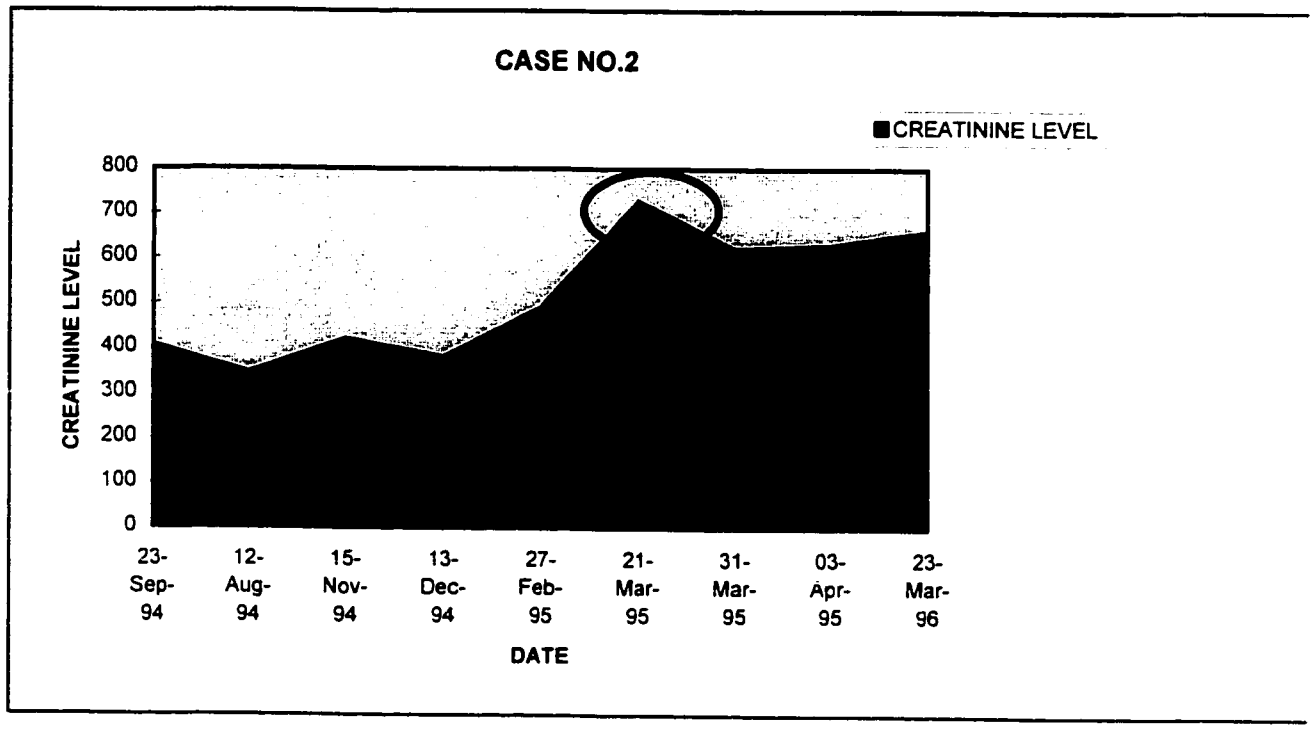


Fig.(3.8b) Follow-up serum creatinine curve

The circle represent the time of graft failure due to renal allograft nephropathy. The patient lost graft function after 195 days of being diagnosed as chronic rejection.

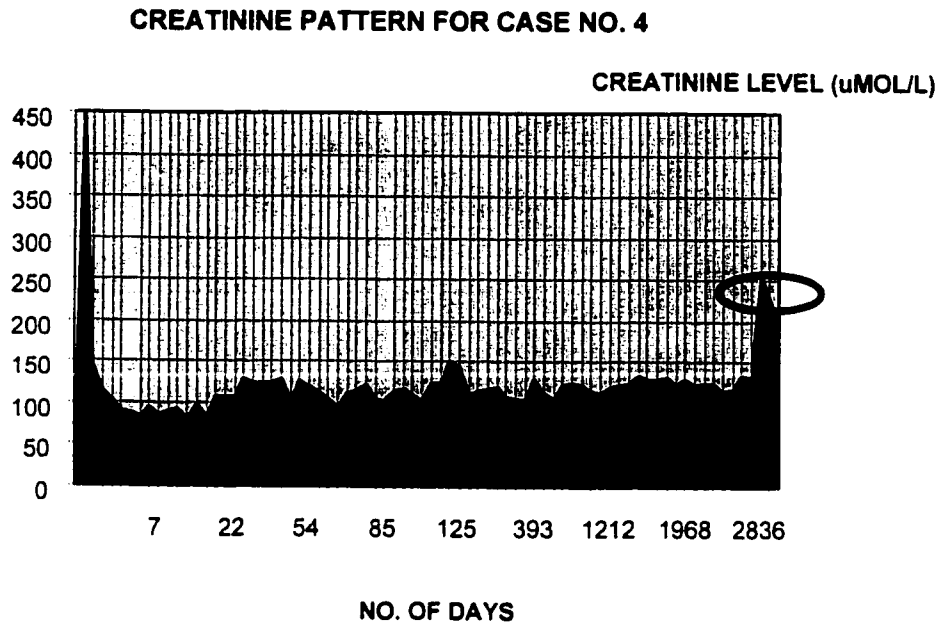


Fig. (3.9) Serum Creatinine level Vs. number of days

Figure (3.9) shows the serum creatinine level with normal fluctuation (base line =150 μMol/L) . Then, at the time of protocl biopsy there was a sudden rise of creatinine level to 250 μMol/L followed by a drop (198 μMol/L) and this patient diagnosed as chronic allograft nephropathy.

The circle represent the time of performing the protocol biopsy .

Prediction of Renal Failure Due to Chronic Rejection:

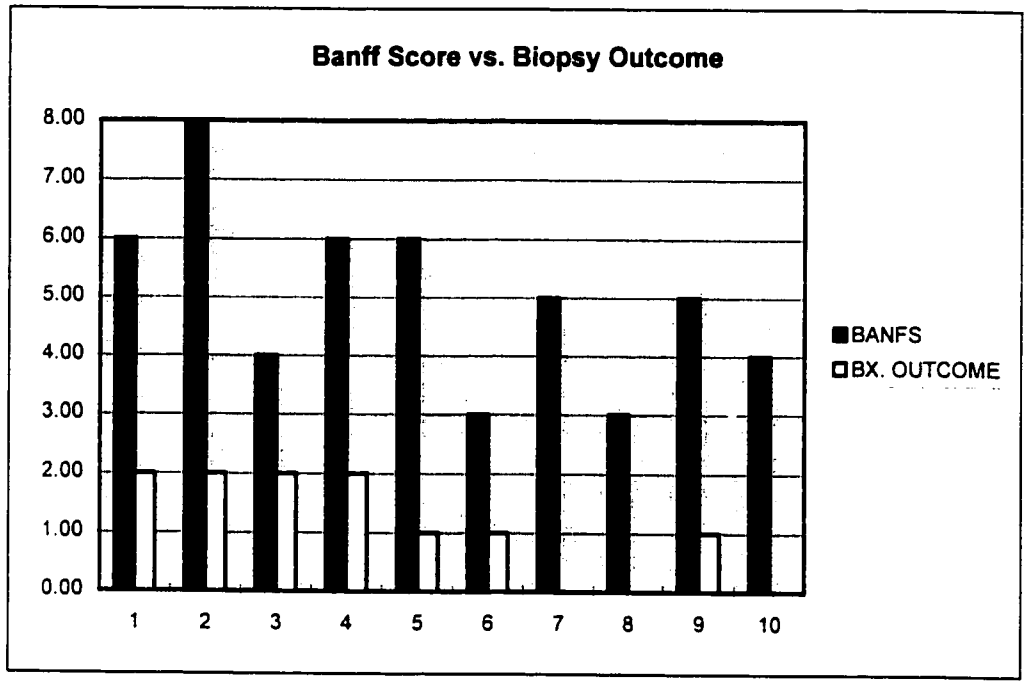


Figure (3.10a)

Figure (3.10a) represents the correlation between Banff score (pathological grading of chronic renal rejection) and clinical progression of chronic renal rejection to renal failure.

This figure shows no correlation between the severity of the pathological lesion estimated by Banff Schema and the progression of renal failure due to chronic allograft nephropathy .

BX OUTCOME:

2 = Failing allograft.
1 = Deterioration of renal function.
0 = No failure.

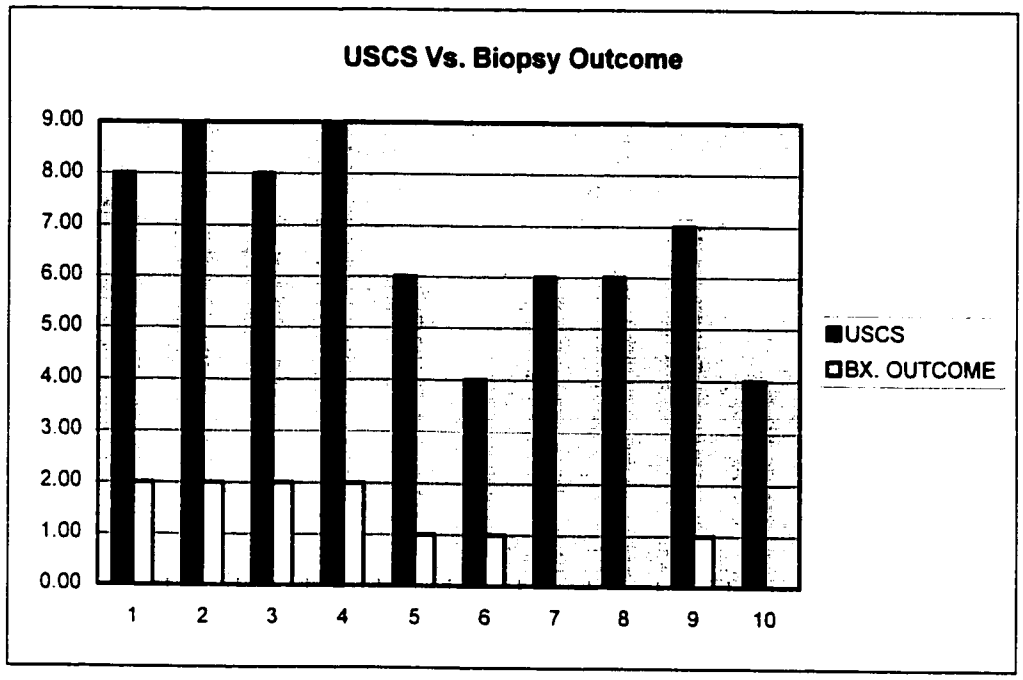


Figure (3.10 b)

Figure (3.10b) represents the correlation between ultrastructural score (ultrastructural grading of chronic renal rejection) and clinical progression of chronic renal rejection to renal failure.

Figure (3.10 b) shows that patients with ultrastructural score of seven or more are likely to end up with renal failure due to chronic rejection. Therefore renal failure due to chronic renal rejection can be predicted by the severity of ultrastructural changes of peritubular capillaries.

References:

1) Sanfilippo, F : Renal transplantation , the pathology of organ transplantation. The Pathology Organ Transplantion. Boston, MA, Butterworth, 1990.

2) Scronick, J, Le Foo, MW, Cicciarelli. Hyperacute rejection and kidney allograft rejection due to antibodies against B cell. Transplantation 1992; 54:61-64.

3) Svalander C, Nyberg, G, Blohme I. Acute early allograft and C3 / microphage phenomenon. Transplant proc 1993; 25:903-904.

4) Ghasemian SR, Light JA, Currier CB, Sasaki T, Aquino A, Rees J, Ward W, Carr J. The significance of the IgG anti-B-cell crossmatch on renal transplant outcome.Clinical Transplant 1997 Oct; 11: 485-7.

5) Rao, KV. Kasis Ke BL, Bloom PM. Acute rejection in late survivors of renal transplantation: Transplantation 1989; 47:290-292.

6) Solez, K, Racusen LC, Marcussen N, Slatnik I, Keown P, Olsen S, Burdick JF. Morphology of ischemic acute renal failure, normal function, and cyclosporine toxicity in cyclosporine treated renal allograft. Recipient Kidney International 1993; 43:1058-1067.

7) Solez K, Axelsen RA, Halloran PF, Hayry P et al. International standardization of criteria for hostologic diagnosis of renal allograft rejection. The Banff working classification of kidney transplant pathology. Kidney International 1993; 44:411 - 422.

8) Nadasdy T, Ormas J, et al. Tubular ultrastructure in rejected human renal allografts. Ultrastructrural Pathology 1988; 12:195 -207.

9) Sibley PK, Rynasiewicz J, et al. Morphology of cyclosporine nephrotoxicity and acute rejection in patients immune suppressed with cyclosporine and prednisone surgery 1983; 94: 225-234.

10) Porter KA, Thomson WB, Owen K, et al. Obliterative vascular changes in four human kidney transplants Br Med J. 1963; 2639-645.

11) Porter KA, Marchioro Tl, Starz TE. Pathological changes in 37 human renal homotransplants treated with immune suppressive drugs. Br J.Urol 1965; 37:250-273.

12) Kincaid-Smith P. Histological diagnosis of rejection of renal allografts in man. Lancet 1967; 2:849-852.

13) Busch GJ, Reynolds FS, Galvanek EG, et al. Human renal allograft , the rate of vascular injury in early allograft rejection. Medicine 1971; 50:29-83 .

14) Alpers CE, Hudkins KL, et al.Expression of vascular adhesion molecule-1 in kidney allograft rejection. Kidney International 1993; 44: 805-816.

15) Halloran PF, Wadgymar A, Ritchie S, et al. Significance of the anti-class I antibody response: I Clinical & pathologic features with anti-class I - mediated rejection. Transplantation 1990; 49:85-91.

16) Halloran PF, Schlaut J, Solez K, Srinivasa NS. The significance of the anti-class I response: II Clinical and pathologic features of renal transplants with anti-class I-like antibody. Transplantation 1992; 53:550-555.

17) Colvin RB, Cohen A, Saiontz C, Bonsib S, Buick M, Burke B, Carter S, Cavallo T, Haas M, Lindbland A, Nast C, Spargo B, Weaver C, Wiss M.Evaluation of pathological criteria for acute renal allograft rejection : Reproducibility, Sensitivity and clinical correlation. J Am Soc Nephrol 1997; 8:1930-1941.

18) Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, Croker BP, Demetris AJ, et al .The Banff 97 Working classification of renal allograft pathology. Kidney International 1999; 55:713-723.

19) Finkelstein FO, Siegel NJ, Bastl C, Forrest JNJ, Kashgarian M. Kidney transplant biopsies in the diagnosis and management of acute rejection.Kidney Int 1976; 10:171-178.

20) Banfi G, Imbasciati E, Tarantino A, Ponticelli C. Prognostic value of renal biopsy in acute rejection of kidney transplantation. Nephron 1981; 28:222-226.

21) Matas AJ, Sibley R, Mauer M, Sutherland DE, Simmons RL, Najarian JS. The vaule of needle renal allograft biopsy : A retrospective study of biopsies performed during putative rejection episodes. Ann Surg 1983; 197:226-237.

22) Solez K, International standardization of criteria for histological diagnosis of chronic rejection in renal allografts. Clinical Transplantation 1994 Jun; 8(3 pt 2) :345-50.

23) Rush DN, Jeffery JR, Gough J. Sequential protocol biopsies in renal transplant patients. Clinico-pathological correlation using the Banff schema. Transplantation.1995 feb 27; 59(4):511-4.

24) Gaber LW, Moore LW, Alloway RR, et al. Clinical applicability of the Banff classification of early renal allograft biopsies. Transplantation Proc 1995; 27:1009

25) Gaber LW, Schroeder T J, Moore LW, Amiri HS, Gaber AO. The correlation of Banff scoring with reversibility of first and recurrent rejection episodes. Transplantation 1996 Jun 27; 61: 1711-1715.

26) Gaber LW, Moore LW, Gaber AO, First MR, Guttmann RD, Pouletty P, Schroeder TJ, Soulillou JP. Utility of standardrized histological classification in the management of acute.1995 Efficacy Endpoints conference. Transplantation 1998 Feb 15; 65 (3) : 376-80.

27) Gaber LW. Role of Renal Allograft Biopsy in Multicenter Clinical Trials in Transplantation .American Journal of Kidney Diseases 1998 June; 31 (6 suppl 1) : S19-S25.

28) Trpkov K , Solez K. Effect of acute tubulitis and vasculitis on allograft function in patients treated with mycophenolate mofetil Banff 97 CD-ROM. Saunders-Churchill;1998.

29) Nickeleit V, Vamvakes EC ,Pascual M , Poletti BJ, Colvin RJ. The prognostic significance of significant arterial lesions in acute allograft rejection. J Am SOC Nephrol 1998; 7:1301-1308.

30) Paul LC . Chronic Rejection of Organ Allografts: Magnitude of the Problem. Transplant Proc 1993; 25:2024-2025.

31) Billingham ME, Cary NRB, Hammond ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection : Heart Rejection Study Group .The International Society for Heart J Heart Transplant 1990; 9:587.

32) Paul LC, Hayry P, Foegh M, Dennis MJ, Mihatsch MJ, Larsson E, Fellstrom B. Diagnostic Criteria for Chronic Rejection/Accelerated Graft Atherosclerosis in Heart and Kidney Transplants: Joint Proposal From the Fourth Alexis Carrel Conference on Chronic Rejection and Accelerated Arteriosclerosis in Transplanted Organs. Transplant Proc 1993; 25:2022-2023.

33) Myers BD, Newton L, Boshkos C, et al. Chronic injury of human renal microvessels with low dose cyclosporine therapy. Transplantation 1988; 46:694.

34) Yilmaz S, Takinen E, Hayry P, Isoniemi H. Protocol Core Biopsy as Intermediate Efficacy End-Point in Chronic Kidney Allograft Rejection 1996 feb; 28 (1): 491-493.

35) Isoniemi H, Nurminen M, Tikkanen M, Von Willebrand E, Krogerus L, Ahonen J, Eklund B, Hockerstedt K, Salmela K, Haryry P. Risk factors predicting chronic rejection of renal allografts. Transplantation 1994; 57:68.

36) Dimeny E, Wahlberg J, Larsson E, Fellstrom B, Can histological findings in early renal allograft biopsies identify patients at risk for chronic vascular rejection ? Clin Transplation 1995; 9:79-84.

37) Nickerson P, Jeffery J, Gough J, Mckenna R, Grimm P, Cheang M, David R. Identification of clinical and histopathological risk factors for diminished renal function 2 years posttransplant. Journal of the American society of Nephrology 1998 Mar; 9(3):482-7.

38) Seron D, Moreso F, Bover J, Condom E, Gil-Vernet S, Caanas C, Fulladosa X,Torras J, Carrera M, Grinyo JM, Alsina J.Early protocol renal allograft biopsies and outcome. Kidney International. 1997 Jan ; 51(1):310-6.

39) Cohen AH, Nast CC. TGF-beta in renal allograft rejection. Miner Electrolyte Metab 1998; 24(2-3):197-201.

40) Horvah LZ, Friess H, Schillin, et al. Altered expression of transforming growth factor-beta S in chronic renal rejection. Kidney Int 1996 Aug; 50(2):489-98.

41) Shihab FS, Yamamoto T, Nast CC, Cohen AH, Noble NA, Gold LI, Border WA. Transforming growth factor-beta and matrix protein expression in acute and chronic rejection of human renal allograft. J Am Soc Nephrol 1995 Aug; 6(2):286-94.

42) Wang Y, Pratt JR, Hartley B, Evans B, Zhang L, Sacks SH. Expression of tissue type plasminogen activator and type 1 plasminogen activator inhibitor, and persistent fibrin deposition in chronic renal allograft failure. Kidney Int 1997 Aug; 52(2):371-7.

43) Shihab FS, Tanner Am, ShaoY, Weffer MI. Expression of TGF-beta 1 and matrix proteins is elevated in rats with chronic rejections. Kidney Int.1996 Dec;50(6): 1904-13.

44) Kerby, JD, Verran DJ, Lou KL, Ding Q, Tagouri Y, Herrera GA, Diethelm Ag, Thompson JA. Immunolocalization of FGF-1 and receptors in human renal allograft vasculopathy associated with chronic rejection.Transplant 1996 Aug 27; 62(4): 467-75.

45) Kerby JD, Luo KL, Ding Q, Tagouri Y, Herrera GA, Diethelm AG, Thompson JA. Immunolocalization of acidic fibroblast growth factor and receptors in tubulointerstitial compartment of chronically rejected human renal allografts.

46) Alpers CE, Davis CL, Barr D, Marsh CL, Hudkins KL. Identification of platelet-derived growth factor A and B chains in human renal vascular rejection. Tansplant Int 1996; 9Suppl 1:S54-7.

47) Pedagogos E, Hewitson TD, Walker RG, Nicholis KM, Becker GJ. Myofilbroblast involvement in chronic transplant rejection. Transplantation 1997 Oct 27;64(8): 1192-7.

48) Guijarro C, Massy ZA, Kasiske BL. Clinical correlation between renal allograft failure and hyperlipidemia. Kidney Int Suppl 1995 Dec; 52:S56-9.

49) van der Dorpel MA, Ghanem H, Rischen-Vos J, Man in't Veld AJ, Jansen H, Weimar W. Low-density lipoprotein oxidation is increased in kidney transplant recipients. Transpl int 1996; 9 Suppl 1:S54-7.

50) Kasiske BL. Clinical correlates to chronic renal allograft rejection. Kidney Int Suppl 997 Dec; 63:S71-4.

51) Brenner BM, Milford EL, et al. Nephron underdosing : A programmed cause of chronic renal allograft failnre. Am J Kidney Dis 1993; 21(suppl2):66-72.

52) Blumke M, Keller H, Fischer J, Eble F, Nausner M, Kirste G. Donor age is a risk factor in cadaver kidney transplantation. Transplantation Proceedings 1992 Dec. 24(6) :2728.

53) Friedrich J, Albrecht KH, Claus M, Eigler FW. The effect of donor and recipient ages on the results of kidney transplantation.Deutche Medizinische Wochenschrift ; 1995 Apr. 120(14):467-71.

54) Alexander JW, Bennett LE, Breen TJ. Effect of donor age on outcome of kidney transplantation. A two-year analysis of transplants reported to the United Network for Organ Sharing Registry. Transplantation 1994 Mar 27; 57(6):871-6.

55) Albrechtsen D, Leivestad T, Sodal G, Bentdal O, Berg KJ, Brekke I, Fauchald P, Flatmark A, Jakobsen A, Lein B, et al.Kidney transplantation in patients older than 70 years of age. Transplantation Proceedings 1995 Feb; 27(1):986-8.

56) Cantarovich D, Giral-Classe M, Le Sant JN, Hourmant M, Dantal J, Blancho G, Karam G, Soulillou JP. Renal transplantation from cadaver donors over 60 years old. Clinical Transplants 1994; 237-42.

57) Kokado Y, Moutabarrik A, Kameoka H, Tanako Y, Takahara S, Ishibashi M, Okuyama A. Impact of donor renal function in kidney transplantation. Transplantation Proceedings 1993 Jun; 25(3):2267-9.

58) Creagh TA, Mclean PA, Donovan MG, Walshe JJ, Murphy DM Older donors and kidney transplantation. Transplant International 1993 Jan; 6(1):39-41.

59) Vianello A, Mastrosimone S, Calconi G, da Porto A, Palminteri G,D'Annibale A, Caldato C, Maresca MC. Influence of donor age on cadaver kidney graft function and survival : univariate and multivariate analyses. Nephron. 1993; 65(4):541-8.

60) Higgins RM, Bevan DJ, Vaughan RW, Phillips AO, Snowden S, Bewick M, Scoble JE, Hendry BM.5-year follow-up of patients successfully transplanted after immunoadsorption to remove anti-HLA antibodies. Nephron 1996; 74(1):53-7.

61) Katznelson S, Gjertson DW, Cecka JM. The effect of race and ethnicity on kidney allograft outcome. Clin Transpl 1995; 379-94.

62) Connolly JK, Dyer PA, Martin S, Parrot NR, Pearson RC, Johnson RW. Importance of minimizing HLA-DR mismatch and cold preservation time in cadaveric renal transplantation. Transplant 1996 Mar 15; 61(5):709-14.

63) Azuma H, Nadeau K, Mackenzie HS, Brenner BM, Tilney NL. Nephron mass modulates the hemodynamic, cellular, and molecular response of the rat renal allograft.Transplantation 1997 Feb 27; 63(4): 519-28

64) Azuma H, Nadeau K, Takada M, Mackenzie HS, Tilney NL.Cellular and molecular predictors of chronic renal dysfunction after initial ischemia/reperfusion injury of a single kidney. Transplantation 1997 Jul 27; 64(2):190-7.

65) Terasaki PI, Gjertson DW, Cecka JM, Takemoto S, Cho YW. Significance of the donor age effect on kidney transplants. Clin Transplant 1997 Oct;11(5 Pt 1): 366-72.

66) Nyberg SL, Manivel JC, Cook ME, Gillingham KJ, Matas AJ, Najarian JS. Grandparents donors in a living related renal transplant program. Clin Transplant 1997 Oct; 11(5 Pt 1):349-53.

67) Almonds PS, Matas A, Gillingham K, Dunn DL, Payne WD. Risk factors for chronic rejection in renal allograft recipients. Transplantation 1993 Apr; 55(4):752-6; discussion 756-7.

68) Matas,A,Chronic rejection in renal transplant recipients—risk factors and correlates. Clinical Transplantation 1994 Jun; 8(3 Pt 2) :332-5.

69) Massy ZA, Guijarro C, Kasiske BL. Clinical predictors of chronic renal allograft rejection. Kidney International - Supplement;1995 Dec;52:S85-8.

70) Laine J, Etelamaki P, Holmberg C, Dunkel L. Apoptotic cell death in human chronic renal allograft rejection. Transplant 1997; Jan 15; 63(1): 101-5.

71) Yokoyama I, Uchida K, Kobayashi T, Tominaga Y, Orihara A, Takagi H. Effects of prolonged delayed graft function on long-term graft outcome in cadaveric kidney transplantation. Clin Transplant 1994 Apr; 8(2 Pt 1): 101-6.

72) Solez K, Alxelsen RA, Halloran PF, Hayry P et al. International Standerization of Criteria for Histological Diagnosis of Renal Allograft Rejection. The Banff Working Classification of Kidney Transplant Pathology. Kidney International 1993; 44:411-422 .

73) Mihatach MJ, Ryffel B, Gudat F. Morphological Criteria of Chronic Rejection : Differential Diagnosis, Including Cyclosporine Nephropathy. Transplant Proceedings 1993; 25: 2031 - 2037.

74) Ramos, E. Recurrent disease in renal allograft. J Am Soc Nephrol 1991; 2:109-121.

75) Campistol JM, Oppenheimer F, Darrell A, et al. Incidencia y caracteristicas de las enfermedades glomerulares postransplante renal. Med Clin (Barc) 1990; 94:761 -765.

76) Shwartz A, Krause PH, Offerman G, et al. Recurrent and de novo renal disease after kidney transplantation with or without cyclosporine A. Am J Kidney Dis 1991; 7:524-531.

77) Sanfilippo F. Renal transplantation, in Sale GE(ed). The Pathology of Organ Transplantation. Boston, MA ,Butterworth, 1990 .

78) Lewis EJ. Recurrent focal sclerosis after renal transplantation. Kidney Int 1982; 22:315-523.

79) Itami N, Akutus Y, Hattori M, et al. Recurrence of nephrotic syndrome in focal segmental glomerulosclerosis after transplantation. Pediatr Nephrol 1990; 4:298-299.

80) Glossak RJ, Cohen AH, Adler S, et al. Primary glomerular diseases, in Brenner BM, Rector FC(eds). The Kidney (ed 3). Philadelphia, PA, Saunders, 1986.

81) Diaz-Tejeiro R, Maduell F, Diez J, et al. Loss of renal graft due to recurrent IgA nephropathy with rapidly progressive course. An unusual clinical evolution. Nephron 1990; 54:341-343.

82) Antignac C, Hinglais N, Gubler MC, et al. De novo membranous glomerulonephritis in renal allografts in children. Clin Nephrol 1988; 84:310-314.

83) Hoistma AJ, Kroon A A, Wetzels JF, et al. Association between ureteral obstruction and de novo membranous nephropathy in renal transplant. Transplant Proc 1990; 22:1388-1389.

84) Tatum AH, Bollinger R R, Sanfilippo F. Rapid serologic diagnosis of serum sickness from antithymocyte globulin therapy using enzyme immunoassay. Transplantation 1984; 38:582-536.

85) Mauer S M, Steffes M W, Connett J, et al. The development of lesions in the glomerular basement membrane and mesangium after transplantation of normal kidneys to diabetic patients. Diabetes 1983;32-948-952.

86) Myers BD, Sibley R, Newton L, et al. The long course of cyclosporine-associated chronic nephropathy. Kidney Int 1988; 33:590

87) Baletta M, Libetta C, Fuiano G, et al. Effect of atwo-month treatment with low oral dose of cyclosporine on renal function. Nephrol Dial Transplant 1991; 6:324.

88) Zaltzman J S, Pei Y, Maurer J, et al. Cyclosporine nephrotoxicity in lung transplant recipients.Transplantation 1992; 54: 875.

89) Mihatsch MJ, Theil G, Rasier V, et al. Alterations in structure and function in a rat model of cyclosporine nephrotoxicity . J Pharmacol Exp Ther 242:749.

90) Besschorner WE, Burdick JF, Solez K. The presence of leu7 reactive lymphocyte in renal allografts undergoing acute rejection. Transplantation 1985;17: 618-622

91) Solez, K, Battaglia D, Fahmy H, Tropkov K. Pathology of kidney transplant rejection. Current opinion in Nephrology and Hypertension 1993; 2:904-911.

92) Mihatch MJ, Ryffel B, Gudat F. Morphological criteria of chronic rejection: differential diagnosis, including cyclosporine nephropathy. Trans Proc 1993; 25(2): 2031-2037.

93) Mihatch MJ, Histolopathology of chronic rejection (abstract) XVII World Congress. The Transplantation Society, Montreal, July 12-17,1998

94) Monga G, Mazzucco G, Novara R, Reale L. Intertubular capillary changes in kidney allografts; an ultrastructural study in patients with transplant glomerulopathy.Ultrastruct Pathol 1990; 14: 201-209.

95) Monga G, Mazzucco G, Messina M, Motta M, Quaranta S, Novara R. Intertubular capillary changes in kidney allografts: a morphological investigation on 61 renal specimens. Mod Pathol 1992: 5: 125-130

96) Ivanyi B, Hansen H, Olsen T S. Postcapillary venule-like transformation of peritubular capillaries in acute renal allograft rejection. Arch Pathol Lab Med 1992: 116: 1062-1067.

97) Ginette Lajoie. Antibody-mediated rejection of human renal allografts: An electron microscopic study of peritubular capillaries. Ultrastructural Pathology1997; 21:235 -242.

98) Drachenberg CB, Steinberger E, Hoehm-Saric E, Heffes A, Klassen DK, Bartlett ST, Papadimitriou JC.Specificity of intertubular capillary changes: comparative ultrastructural studies in renal allograft and native kidneys. Ultrastruct Pathol 1997 May-Jun; 21(3): 227-33.

99) Forbes RDC, Guttmann RD, Gomersall M, Hibberd J. A controlled serial ultrastructural tracer study of first-set cardiac allograft rejection in the rat. Am J Pathol 1983;111;184-196.

100)Dvorak H F, Mihm M C, Dvorak AM, Barnes B A, Manseau E J, Galli S J. Rejection of first-set skin allografts in man. J Exp Med 1979; 150:322-337.

101)Matsumoto Y, McCaughan GW, Painter DM, Bishop GA. Evidence that portal tract microvascular destruction precedes bile duct loss in human liver allograft rejection. Transplantation 1993; 56:69-75.

102)Basadonna GP, Matas AJ, Gillingham KJ. Early Versus Late Acute Renal Allograft Rejection : Impact on Chronic Rejection .Transplantaion 1993; 55:993-955.

103)Almond PS , Matas AJ, Gillingham K. Risk Factors of Chronic Rejection in Renal Allograft Recipients . Transplantaion 1993 ; 55:752-757 .

104) Lindholm A, Ohlman S, Albrechtsen D, et al. The impact of acute rejection episodes on long- term graft function and outcome in 1347 primary renal transplants treated by 3 cyclosporine regimens. Transplantation 1993; 56:307.

105) Pekka Hayry, et al. Chronic renal allograft rejection can be predicted by area under serum creatinine vs. time curve . Kidney International 1995, July; 48(1:251-8).

106) Sedmak DD, Orosz CG. The role vascular endothelial cells in transplantation. Ach Pathol Lab Med. 1991; 115: 260-265.

107)Bishop GA, Waugh JA, Landers DV, Krensky AM, Hall BM. Microvascular destruction in renal transplant rejection. Transplantion 1989: 48:408-414.

108) Anderson ND,Wyllie RG, Shaker IJ, Pathogenesis of vascular injury in rejecting rat allografts. John Hopkins Med J. 1977; 141: 135-147.

109) Varcko R, Benditt EP. Capillary basal lamina thickening : its relationship to endothelial cell death and replacement. J Cell Biol. 1970; 47: 281-285.