Age Related Histological Changes in Rat Kidney

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Histological changes in rat kidney associated with growth and aging were assessed from day one after delivery to thirty months of age. Growth was associated with increase in body and total kidney weight. Different parts of nephron showed an increase in size with growth . In aging rats there was compensatory hypertrophy of renal corpuscle, focal atrophy of proximal and distal convoluted tubules with intra tubular cast and cellular infiltration.
Key words: Age, histological changes, rat kidney

Aging is associated with progressive changes in renal structure and function and it becomes difficult to identify age related changes from those produced by drugs[1]. The mechanism involved is a combined affect of extrinsic influences and an intrinsic molecular programme of cellular aging, in which predetermined sequence of events ultimately leads to senescence. Progressive reduction in anti oxidative defense mechanism (vitamin E, glutathione peroxide) favors such cellular changes[2] Animal studies have shown lower resistance to oxidative stress indicated by increased levels of lipid peroxides in brain and peripheral organs in elderly[3]. Invitro studies suggest that in aging kidneys telomeres shorten in an age dependent manner, particularly in renal cortex because aging cells have diminished capacity for repair of damaged chromosomes[4].

Aging rats show more apoptosis in their tubular epithelial cells, tubulointerstitial fibrosis, interstitial inflammation and fibroblast activation. Three stages of tubulointerstitial injury were observed by GreenFled[5].Stage 1: Mild fibrosis with atrophy and cast in medullary thick ascending limb of loop of henle. Stage 2: Extensive fibrosis and atrophy with large cast formation. Stage 3: Maximal urinary osmolality is decreased in correlation with the stage of tubulointerstitial injury.

GFR decline with age so is renal blood flow, with redistribution of blood flow from cortex to medulla and increased vascular resistance. There is atrophy of afferent and efferent arterioles and formation of direct channel between afferent and efferent arterioles contributing to maintenance of medullary blood flow[6]. There is degeneration of cortical glomeruli, thickening of glomerular and proximal convoluted tubular basement membrane, mesengial proliferation,and glomerulosclerosis[7] Glomerular atrophy is followed by compensatory hypertrophy of other glomeruli[8].

Advanced glycogen end products (AGE) and lipofuscin pigment in epithelium especially in atrophied nephron increases with age and contributes to age related pathology. In early stages of renal aging injury to medullary tubules may be more prevalent than injury to glomeruli, which is responsible for reduction in concentrating ability and impairment in sodium excretion, so, elderly are more prone to diseases such as hypertension and diabetes mellitus. Protein uria (specially albumin uria) is the most striking change in renal function of aging rats and correlates with severity of age related glomerular pathology[9,10].

Age related changes can be slowed down by continuous light exercise, restricting food intake and addition of vitamin E to food. This also reduces protein uria and glomerular hypertrophy[11].

Aims and objectives:
Aim of this study was to asses histological changes in rat kidney during growth and aging so that in experimental animals affects of drugs can be differentiated from those induced by.aging.

Materials and methods:
This study was carried out in Post Graduate Medical Institute, Lahore.Adult rats were obtained from N.I.H, Islamabad.They were kept in the animal house of PGMI and were sacrificed at the age of 30 months.It was labeled group V . Young rats were delivered in PGMI and were divided into 4 groups, (I-IV), each group comprised of three animals

Table 1 Grouping of experimental animals.

Group	No of animals	Age of animal when sacrificed
I	3	1st day after birth
II	3	One week
III	3	3 months
IV	3	12 months
V	3	30 months

All animals were provided with food and water ad libitum. Care was taken for optimal light and temperature. Urine strips were used to check urinary proteins. Blood samples were collected for renal functions. Animals were weighed and sacrificed according to table No. 1. Kidneys were obtained, blotted, weighed and were fixed in formal saline, Paraffin blocks were made and four micron thick sections were cut. Sections were stained with Haematoxylin and Eosin. For basement membrane Periodic acid Schiff reagent was used. Slides were studied under light microscope. Comparison was made between different age groups. For degree of glomerular sclerosis 50

sequential glomeruli / slide were studied and following scoring system was developed. Grade 0= 0% sclerosis, Grade 1=25%, Grade 2=50%, Grade 3=75% and Grade 4= global sclerosis. For tubulointerstitial injury Green Field staging system was used.

Following parameters were observed.

Renal stroma for Fibrosis Cellular infiltration

Renal parenchyma

Glomeruli for sclerosis and diameter.

Tubules for diameter and number of nuclei/ ts and cast

Renal functions blood urea and creatinin

Protein uria.

Tissue body wt index was calculated by the following formula

$$TBI = \frac{\text{kidney wt}}{\text{Body wt}} \times 100$$

Results:

Sprague dawley rats had an increase in weight from an average of 5.6 g to153 g in first year .In next 18 months it became 550 g. There was no change in their eating habits but they became aggressive at about two years of age, so they were separated in individual cages. Their fertility was not affected.

Total kidney weight (rt +lt) was 0.091gm at birth, which increased to 4.5grams at 30 months of age. Kidney size increased from 3 × 1.5mm to 2.3 × 1.2cm at 30 months (Table 2). Tissue body weight index was 1.6 at birth and 0.8 at 30 months. Renal function tests were performed in young rats (12 weeks) and old rats (30 months), which included serum urea, creatinin and urinary proteins They were of higher normal value

Microscopic examination of kidney

Glomerular changes:

In newborn rats the sub capsular cortex was still undifferentiated which included both renal corpuscles and tubules. (Fig 1)

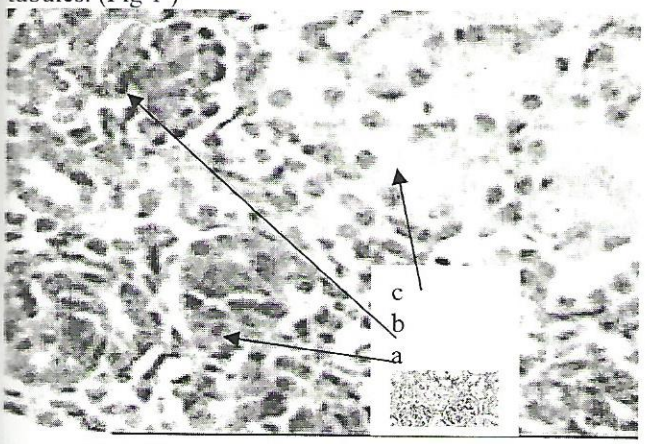

Fig-1 Photo micrograph of new born rat kidney Showing Undifferentiated renal corpuscle in subcapsular region (a), developed renal corpuscle (b),and proximal convoluted tubule (c). H&E (100x)

All structures in juxtamedullary region were very well differentiated. Average measurements of renal corpuscle, PCT and DCT are shown in table 3.

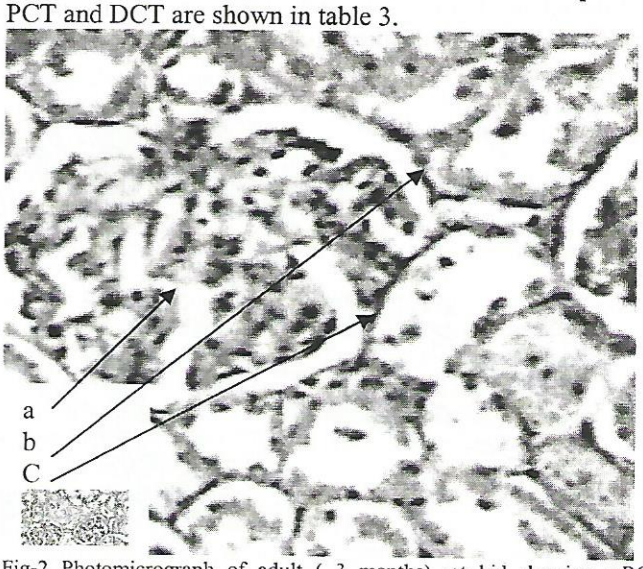

Fig-2 Photomicrograph of adult (3 months) rat kid showing a;RC ,b;PCT, c ;DCT H&E X 400

Although glomerular density decreased in elderly rats, no marked glomerulosclerosis was observed. Glomerular capillary basement membrane and Bowman's capsule was normal in young rats but slight thickening saw observed in elderly rats in 2% of the glomeruli (Fig 3)

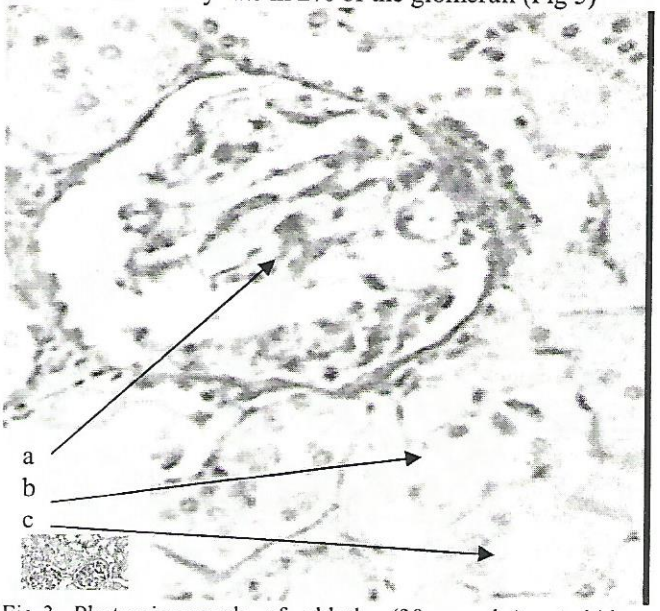

Fig-3 Photomicrograph of elderly (30 months) rat kidney showing a; thickening of glomerular BM b;DCT c; PCT PAS X 400

Renal tubules:

In postnatal rats, tubules in sub cortical region were still in the process of differenciation. In all other groups they were very well developed. Average diameter of renal tubules is given in table 3 .In PCT the numbers of nuclei/cross section were 6-7 .In elderly rats it showed some pyknosis.

Epithelial thickness was 10 micron in newborn and 14 in adult rats; Brush border was very well preserved in most of the tubules but more than20% tubules showed epithelial atrophy , tubular dilatation.and early intra tubular cast in elderly rats. (Fig 4)

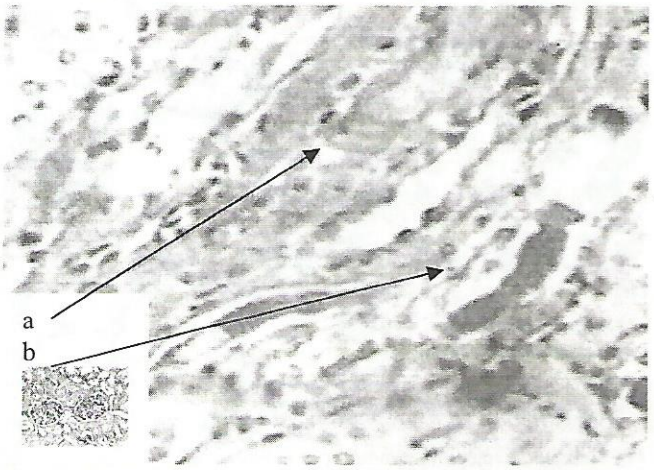

Fig-4 Photomicrograph of elderly rat kidney showing a;Interstitial fiberosis ,b; tubular cast

An average number of 7 to 8 nuclei / cross section were found in DCT. The epithelial thickness was 6 micron in newborn rats & 8 in adults. Brush border was not prominent.

Mild focal atrophy was seen in DCT with tubular hemorrhages and early intra tubular cast in twelve months and thirty months rats (fig 4 & 5).

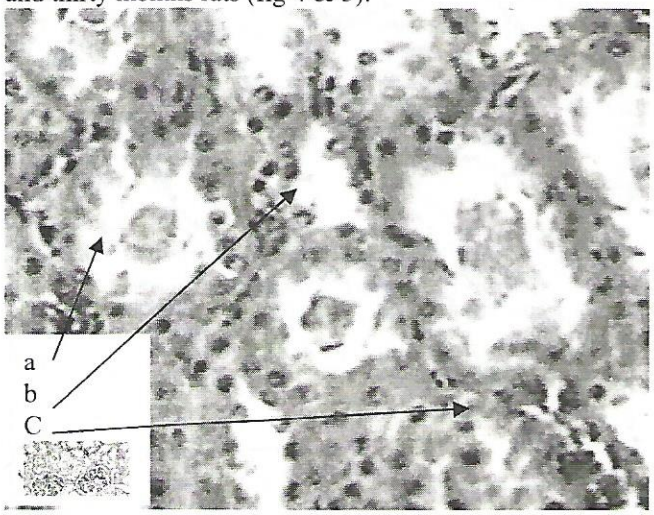

Fig-5 Photomicrograph of elderly rat kidney showing A; PCT with intra tubular cast , b; DCT., c: peritubular haemorrhages

There was thickening and splitting of basement membrane and widening of interstitium with fibrosis. There were multi focal areas of inflammatory infiltrate, consisting of lymphocytes predominantly but mononuclear cells were also present (Fig 6)

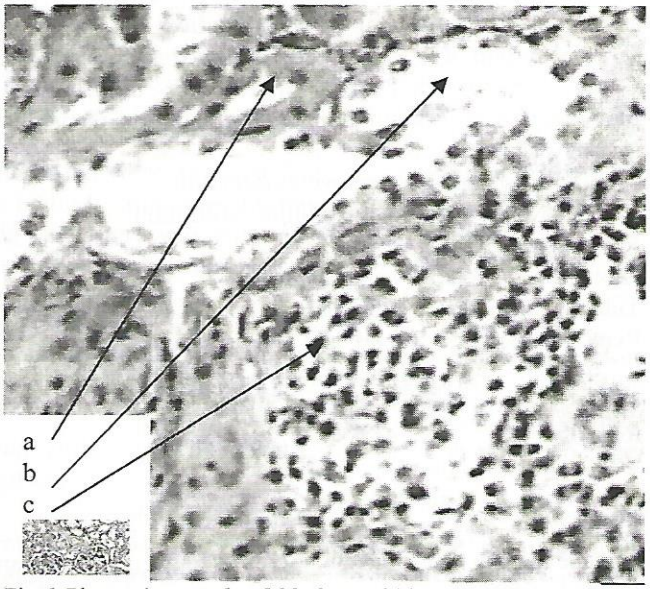

Fig-6 Photomicrograph ofelderly rat kidney showing a; PCT ,b ;DCT, c ;cellular infilteration.

Renal Medulla:

Rats have unilobed kidney, so only one papilla was seen projecting in the renal pelvis. There was focal fibrosis resulting in widening of interstitium. Tubular and peritubular hemorrhages were also found in medulla. Inflammatory infiltrate was especially more dominant in medulla.

Physiological Parameters:

. Blood urea and creatinine levels were of higher normal value Urinary proteins were doubled than in adult rats.

Table 2: Comparison of body wt, kidney wt and size in various age groups of rats

Group	Body wt in grams	Total Kidney wt in grams	Kidney/ Body wt x 100	Kidney Size in mm
1.new born	5.6	0.091	1.6	3,1.5
11.1 wk	8.5	0.112	1.3	9,5
111.12 wks	109	1.22	1.1	16,9
1V.12 mo	153	1.47	1.07	19,10
V .30 mo	550	4.5	0.8	20,12

Table 3 Changes in renal parenchyma in various age groups of rats

Gp	PCT		DCT		Glomerular (diameter in μ	Glomerulo-sclerosis
	Dia	cast (μ)	Dia	cast (μ)		
I	30	nil	23	nil	40	Nil
II	30	nil	25	Nil	46	nil
III	35	nil	30	Nil	53	nil
IV	40	+	30	Nil	89	G1
V	45	++	30	nil	110	G1

Table 4 Changes in renal stroma in various age groups of rats

Group	Cortex			Medulla		
	Fib-rosis	Cellu-lar infilt-ration	Blood vessels	fibrosis	Cellular Infilterat	Blood vessels
I.new born	nil	nil	Normal	nil	nil	normal
II.on e wk	nil	nil	Normal	nil	nil	Normal
III.1 2 wks	nil	nil	Normal	nil	nil	Normal
Iv 12 mo	+	+	Normal	+	+	Normal
V .30 mo	+	++	Normal	+	+++	normal

Discussion:

Increasing age produces behavioral, functional and structural changes in the body, as shown in this study. These changes need to be differentiated from those produced by disease and induced by drugs. Old aged people are vulnerable to many diseases e.g. diabetes and hypertension. These diseases and drugs may accelerate such age related changes. Care must be taken in selecting a drug and calculating its dose for elderly.

Body and kidney wt in rats increased with age, indicating growth. A reduction in tissue, body wt index, however is a marker for a relative decrease in kidney wt in elderly. (Table 2)

All parts of nephron in the renal parenchyma showed an increase in size with increasing age but increase in the size of renal corpuscle was out of proportion to other parts .This is an indication of compensatory hypertrophy of renal corpuscles .Age related increase in renal corpuscle without glomerulosclerosis is also noted by Laurent et al in 1999 and Z Green Feld in 1997. Dhallu et al also found glomerular hypertrophy in elderly rats in 1991 with tubular dilatation and itra tubular cast in both renal cortex and medulla. In our study the major finding was inflammatory infilterate especialy in renal medulla as observed by Susan et al in 1998. The inflammatory infilterate was present in slides obtained from all elderly rats(30 months)with interstitial fibrosis and tubular dilatation .The twelve months rats also showed inflammatory infilterate but intensity was less as compared to elderly rats .NO young rats (3 months) showed infilterate ($P<0.001$) Protein urea was present in young rats, it increased significantly in elderly rats ($P<0.001$) .All other renal functions were towards higher normal values. Results go well with extent of injury observed in renal parenchyma .

Conclusion:

Glomerulosclerosis is not a dominent feature of aging kidney.Tubular injury and interstitial fibrosis starts in twelve months rats and increases with age . It is associated with inflammatory infilterate, indicating interstitial fiberosis as an active process.

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