Hyperlipidemia Aggravates Renal Disease in Bacteremic Male Albino Rats

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Abstract—Electron microscopic study is very essential in assessing the nature and state of the tissues. It is used widely to examine the visceral organs in various diseases, infections and disorders. It provides the required insight and details about the possible malfunctioning of the disease or infection. Male adult albino rats with hyperlipidaemia were taken and test group was infected with Escherichia coli. Abnormalities in lipid metabolism appear to play a pathogenic role in progressive renal disease. The control group was not infected with any clinical pathogen. The electron microscopic examination was carried out to determine the effect of infection in the experimental groups. Kidney was the visceral organ which was used for the study. Difference in case of infected male rats when compared with control group rats on the level of electron microscope can be further extended in the case of other clinical pathogenic infections which could lead to interesting results. Hyperlipidemic-bacteremic group showed increased chronic damage in renal tissue. The deleterious effects of hyperlipidemia with its pathogenetic mechanisms, however, leading to an increase in inflammatory mediators in lipid-induced tubulointerstitial degeneration and the relationship between glomerular and tubulointerstitial damage in this case have not been examined before by the electron microscope. The present study was conducted to find out the role of hyperlipidemia and inflammation caused by E coli in the alteration of kidney tissue by the electron microscope in male albino rats.

Keywords—Escherichia coli, Glomerulus Hyperlipidaemia, Tubulointerstitial tissue.

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I. INTRODUCTION

Impaired kidney function is associated with increased risk for cardiovascular disease[1-2] and may progress over time to end-stage renal disease. Abnormal lipid metabolism has been implicated as a possible cause of these complications[3-4]. Moderate chronic kidney disease (CKD) is associated with elevated triglyceride and diminished HDL cholesterol concentrations [3-8]. Total and LDL cholesterol concentrations have generally been reported to be unaltered in the setting of CKD [5-7]; however, conventional lipid measurements do not fully capture relevant changes in lipid distribution, particularly differences in LDL particle number and size [3-4]. Moreover, lipid and lipoprotein concentrations have not been described at the earliest stages of kidney disease, when the pathophysiologic processes that lead to atherosclerosis and CKD could already be developing.

Ultrastructural pathology is an essential tool in assessing the physical nature of various living tissues. The ultrastructural examination serves as a great tool in determining the infection state and provides the basis for its treatment. Various infections that are possible in the cell are bacterial, fungal and viral in origin. In this, the bacterial infections are the most common and most easy to cure also[2]. The organs that are examined in case of each infecting agent is different and is case sensitive. Kidney is important in its own way as it determines the inorganic concentration of the body. Any examination technique involved in kidney should ensure that the tissue damage is minimized to the best possible extent without which proper results cannot be ensured. Hyperlipidemia may be familial or secondary to several conditions like high-lipid diet, hypothyroidism and liver dysfunction[9-11]. Hyperlipidemia induced by metabolic conditions cause lipid accumulation mostly in kidney tissue with alterations in kidney cortex[4]. However, the effect of isolated hyperlipidemia and /or other risk factors like inflammation with Escherichia coli on kidney cortex to assess susceptibility of infection alone or in combination with hyperlipidemia has not been studied yet before.

The lipids affect not only glomeruli but also the tubulointerstitium [12]. The lipid-induced tubulointerstitial
damage is of special pathophysiological importance as Risdon et al. [13] and Mackensen-Haen et al. [14] have shown that tubules and interstitium are major determinants of renal excretory function. Interstitial fibrosis and tubular atrophy have been documented in both female and male hypercholesterolemic rats without primary glomerular disease [12, 13]. Renal mRNA of the profibrogenic cytokine, transforming growth factor-β1 (TGF-β1), and the chemokine, monocyte chemoattractant protein-1 (MCP-1), were found to be increased in these experiments. The pathogenetic mechanisms, however, leading to an increase inflammatory mediators in lipid-induced tubulointerstitial fibrosis and the relationship between glomerular and tubulointerstitial damage have not been examined in detail. Endotoxin, a component of the wall of Gram-negative bacteria with significant proinflammatory properties, is a potent inflammatory agents that have many physiologic and biochemical functions in vivo, including increased circulating acute phase proteins [16]. Injection of endotoxins into rats resulted in hyperlipidemia [17]. Pro-atherogenic effects of endotoxin infusion were observed in cholesterol-fed piglets and rabbits [18,19]. Weekly injections of rabbits with endotoxins significantly elevated atherosclerosis as evidenced by increased aortic lesion area, although no effect of endotoxins was observed on the serum triglycerides or serum low-density lipoprotein (LDL) cholesterol levels [20]. An imbalance in the generation of oxidants and antioxidants seem to have a vital role in the pathophysiology of atherosclerosis. The expression of the extracellular form of superoxide dismutase (SOD) increases with the severity of atherosclerosis and is associated with an enhancement of SOD activity [21]. Because it is now postulated that each of these reactive species activates specific cell-signaling pathways, the importance of this enzyme in regulating cellular responses at sites of inflammation is evident. Emerging evidence indicates that ROS are important risk factors in the pathogenesis of kidney diseases if the anti-oxidant system is impaired. A recent study showed that hyperlipidemia significantly increased total cholesterol, triglycerides, low density lipoprotein (LDL) and homocysteine levels, whereas decreased high density lipoprotein cholesterol (HDL) levels. Moreover, hyperlipidemia induced mild oxidative stress in terms of elevated levels of malondialdehyde (MDA) and nitric oxide (NO) and decreased level of reduced glutathione (GSH) in blood [22]. The present study was conducted to find out the role of hyperlipidemia with inflammation caused by E. coli alone or in combination in the alteration of kidney tissue by electron microscopic study in male albino rats.

II- Materials and Methods

Male adult albino rats (150-200 g) were provided from the breeding center of National Organization for Drug Control and Research (NODCAR) and kept for a week for acclimatization under normal conditions and constant temperature with ad libitum of water and food. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Organization for Drug Control and Research Egypt. All chemicals used were of analytical grade. Solution of pathogenic strain of Escherichia coli(E. coli) bacteria was provided by microbiology department NODCAR. The solution was diluted with saline to colony forming units 2x10^7 CFU/ml. Each rat intraperitoneally administered 0.5 ml containing 10^7CFU. A group of animals fed normal diet served as normal control group (group I n=10).

2.1. Induction of hyperlipidemia (group II):- Hyperlipidemia in rats was done according to the method of the author [23]. In briefly, hyperlipidemia was induced by feeding the animals high-fat diet (40%) fat / cholesterol (5%) for two months. The high-fat diet contained cholic acid (0.35%) to enhance the enteral absorption of lipids. The occurrence of hyperlipidemia was determined by measuring lipid profile (total cholesterol, triglycerides and HDL). Animals with hyperlipidemia were the only used (n=10).

2.2. Experimental design (group III & IV) (n=10) :- Bacteremic animals (group III) normal diet-fed animals were injected with single dose of E.Coli (107 CFU/rat) and kept for two weeks (n=20). This group is comprised of twenty rat, because from previous studies the mortality rate of bacteremia amounted to 50%.

- Hyperlipidemic- bacteremic animals (group IV) (n=10): twenty hyperlipidemic rats (as in group II) injected with single dose of (107 CFU/rat) E.Coli and kept for two weeks after feeding high fat diet for two months. The animals were sacrificed by decapitation. Ultrathin sections from the kidney cortex were processed for electron microscopic examination.

2.3. Electron microscopic study:-

For electron microscopic examination, ultrathin sections (60 nm in thickness) were cut on the RMC MT-7 ultramicrotome by using a diamond knife. Doubly stained sections with uranyl acetate and lead citrate were examined under a Joel EX 1200 Transmission Electron Microscope at the central lab, Ain Shams University and King Saud university.

III- Results

3-1 Control group (Fig.1 a-f)

The renal corpuscle consists of two parts, a thin walled cup-like expansion, the “Bowman’s capsule” and a lobulated tuft of capillaries, the “glomerulus”. The outer wall of Bowman’s capsule, or parietal layer, is formed of simple squamous epithelium which bulges into the urinary space in the region of their nuclei (Fig. 1 a). The inner wall of the capsule is called the visceral, or podocyte, formed of specialized cells called podocytes which have a complex shape (Fig.1 b) Each podocyte has several processes which give rise to numerous secondary processes known as “pedicles”. These processes rest upon the basement membrane of the glomerulus leaving narrow slits between them called filtration slits. Each glomerulus capillary is covered with the “basement membrane” or basal lamina (Fig.1a & b). This lamina is lined by highly attenuated
endothelial cells perforated by narrow pores or fenestrae. Each cell has a single oval nucleus usually located in the part of the cell which is attached by a stalk to the glomerulus. The axial portion in the hilus of the glomerular capillaries has certain cells, known as intercapillary or mesangial cells. These cells are separated from the endothelial cells by an amorphous mesangial matrix. The proximal convoluted tubules (PCTs) are distinguished from the distal convoluted tubules by their narrow lumina, and large diameters, and are lined by a single layer of cuboidal cells. The proximal convoluted tubules have an elaborate shape, well developed microvilli (or brush border) along their lumina, an active endocytotic apparatus, and many spherical or elongated mitochondria (Fig. Ic). The nuclei of the cells are relatively large, mostly euchromatic with prominent nucleoli and always lying at the basal portions of the cells (Fig. Ic &d). Distal convoluted tubule cells (DCTs) appeared cuboidal, with a few, short apical microvilli. Mitochondria are not interposed between the nucleus and the apical membrane, but do fill the compartment between the basal infoldings (Fig. le &f). The nuclei are relatively large and their heterochromatin appears always attached to the nuclear membrane (Fig. 1 e). They are located in the apical part of the cytoplasm.

3-2 Hyperlipidemic group II (Fig. IIa-f);
After two months of feeding the animals high-fat diet [(40%) fat/cholesterol (5%)], the renal corpuscles exhibited irregular appearance and showed a prominent increase in the number of mesangial cells (Fig. Ila &b). The endothelial cells of the glomerular capillaries displayed signs of degeneration. Their nuclei became small and darkly stained (Fig. I1a &c). Foot processes of podooytes were frequently fused and sometimes destructed forming infrequent breaks (Fig. I lc). Numerous lipid droplets were observed particularly in proximal convoluted tubules. Proximal and distal convoluted tubules exhibited vacuolated cytoplasm and fragmentation of mitochondria as a respond to hyperlipidemia (Figs. 11d &e). Orthodoxy configuration of mitochondria were evident in distal convoluted tubule indicating a low level of oxidative phosphorylation (Fig. 11 f). Basal lamina of parietal epithelium and distal convoluted tubules displayed obvious thickening (Figs. I1e &f). Margination of heterochromatin and irregularity of nuclear envelope were obvious in all nuclei of all types of cells (parietal, visceral, mesangial, endothelial).

3-E. Coli treated group III
(Fig. 111 a-f)
The electron micrographs of the third group revealed altered renal cortex of rats treated with E. coli. Widened intercellular space and increased peripheral migration, besides, interposition of mesangial cells, expanded mesangial matrix and accumulation of infiltration in the lumen of capillaries (Fig. IIIa, b &f). The nuclear changes in all types of cells (parietal, visceral, endothelium, mesangial, proximal and distal convoluted tubules) were as follows: margination of heterochromatin, pyknosis, karyorrhexis and finally karyolysis in cells undergoing necrotic degeneration. Loss or effacement of food process of visceral epithelium and continous granular density in glomerular basement membrane were obvious more than lipemic rats (Fig. IIIc). A considerable amount of brush border and the microvilli debris was observed in the lumen. Moreover, disappearance of basal infoldings and fragmentation of mitochondria into spherical forms in both proximal and distal convoluted tubule cells were obviously seen which were considered as indication to pathological condition. (Fig. IIIg &h).

3-4 Hyperlipidemic-bacteremic group IV (Fig. 1V a-f)
After two months of feeding the animals high-fat diet followed by injection with single dose of E. Coli (107 CFU/rat) for two weeks, most of the renal corpuscles and renal tubules were severely affected than group II & III. Glomerulus with approximately complete effacement of foot processes of podocytes was observed and blood capillaries displayed hypertrophied endothelial cells, with intense heterochromatin and irregular nuclear envelope (Fig. IVa-d). In addition, mesangial cells as well as visceral epithelium revealed irregular nuclear envelope (Fig. IVa &c). Loss of normal fenestration of endothelial cells and loss of differentiation of the three layers of glomerular basement membrane were evident. Increased lysosomes of proximal and distal convoluted tubular cells with shorten or loss of microvilli of proximal tubular cells were more frequently increased than GII and GIII (Fig. IV e &f). Distal convoluted tubules appeared hypertrophied and displayed larger irregular nuclei. Sever fragmentation (rounded) as well as wooly focial densities in the mitochondria were observed in distal convoluted tubules (considered to be evidence of irreversible cell injury) (Fig. IVg). Besides, complete loss of basal infoldings and thickened basal lamina were more pronounced in distal convoluted tubules of GIV than GII & GIII (Fig. IV h).

IV- DISCUSSION

Hyperlipidemia is characterized by elevated serum levels of triglycerides, cholesterol or both[24]. It causes lipid accumulation in various tissues such as liver, kidney, muscle, arterial vessel wall and pancreas[25,26]. In our experimental study, we examined the effect of diet-induced hyperlipidemia on the kidney tissue of male bacteremic rats. Our results showed that hyperlipidemia (including hypercholesterolemia and hypertriglyceridemia) caused moderate to severe degrees of intracellular lipid accumulation in major kidney tissue. Furthermore, the renal corpuscles exhibited irregular appearance and showed a prominent increase in the number of mesangial cells (Fig. 11a &b). The endothelial cells of the glomerular capillaries displayed signs of degeneration. Their nuclei became small and darkly stained (Fig. 11a &c). Foot processes of podocytes were frequently fused and sometimes destructed forming infrequent breaks (Fig. 11c). Numerous lipid droplets were observed particularly in proximal convoluted tubules. Moreover, Proximal and distal convoluted tubules exhibited vacuolated cytoplasm and fragmented mitochondria as a respond to hyperlipidemia (Figs. 11d &e). Pyknotic configuration of mitochondria were evident in distal convoluted tubule indicating a low level of oxidative phosphorylation[27] (Fig. 11f). Besides, the basal lamina of parietal epithelium and distal convoluted tubules displayed
obvious thickening (Figs. 11 e&f). In addition, margination of heterochromatin and the irregularity of the nuclear envelope which were obvious in this work in nearly most nuclei of parietal, visceral, mesangial and endothelium cells thus confirming the result for the author [28]" who studied the changes in renal glomeruli during autolysis. A number of studies showed that dietary lipids could modify the composition of the structural lipids of cell membranes and the fluidity of lipid bilayer. It is established that these changes can alter some of the membrane functions, such as transport, receptor characteristics and activities of membrane-associated enzymes[29]. Adenylate cyclase (AC) system is important in cellular signaling and it is sensitive to the change in the composition of dietary lipids[29&30]. The authors [30] showed that feeding a diet rich in cholesterol could decrease AC activity and membrane fluidity. In our study, although membrane fluidity and AC activity were not examined but increased cytoplasmic lipid accumulation may be due to intake of excessive lipid and/or the alteration of the composition of structural membrane lipids. In agreement to the present results, a recent study showed that feeding of albino rats with high fat diet increased atherogenic indices and induced vascular endothelial dysfunction in isolated aorta of atherogenic-diet rats [22&31]. Furthermore, feeding of rats with high fat diet and a single dose of vitamin D produced atherosclerosis in Sprague-Dawley rats, and induced hemorrhheological and histopathological abnormalities in the atherogenic diet fed rat model [32]. Moreover, it is likely that the elevated plasma viscosity might constitute a risk factor in hyperlipidemic subjects [33]. Other studies indicated that hyperlipidemia increased the levels of lipid parameters and induces oxidative stress that initiated atherosclerosis [34]. The present ultrastructural alterations of hyperlipidemic rats in comparison to control group might be due to accumulation of fats in the kidney tissue and its consequences on vascular endothelial dysfunction. The pronounced aggravation of tubulointerstitial damage by hyperlipidemia, especially noticeable in group IV nephritic rats, was accompanied by a dramatic increase in the number of lysosomes of proximal and distal convoluted tubular cells. On the other hand, bacteremic group exhibited ultrastrutural malformation nearly equals hyperlipidemia-bacteremia group that might indicate that hyperlipidemia alone may have minor effect. Also, this might suggest that inflammation plays an essential role in the initiation and progression of atherogenesis in presence or in absence of hyperlipidemia[34]. Consistently, several studies indicated that inflammation causes abrasion of the overlying endothelium of the blood vessels through the exposure to the immune cell monocytes/macrophages and deposition of LDL-cholesterol leading to arteries stenosis even in normal lipid profile individuals [35].

It has been reported that drug disposition is altered in some disease states, notably those associated with functional changes in the kidney, which plays a crucial role in drug and metabolite excretion. Many authors have extensively studied changes in the renal excretion of organic anion drugs in cases of renal failure and insufficiency associated with various disease states, especially endotoxemia induced by a gram-negative bacterial infection[36 &37]. *Escherichia coli* infection induces colonization of the bowel and production of powerful Shiga-like toxins (SLTs), which are thought to enter the circulation system and to cause injury to target endothelial cells in various organs, such as the renal glomeruli and the gastrointestinal tract[38&39]. However, what and how therapy with antimicrobial agents should be done in the treatment of this infection has not yet been clinically clarified. Relevant animal models for *E. coli* infection are needed to study the different states of *E. coli* infectious disease in humans because of the difficulties associated with conducting clinical trials with humans. Our electron micrographs of the third group revealed altered renal cortex of rat treated with *E. coli*. Widened intercellular space and increased peripheral migration, besides, interposition of mesangial cells, expanded mesangial matrix and accumulation of infiltration in the lumen of capillaries. (Fig.4a,b&f). The nuclear changes were evident in all types of cells (parietal, visceral, endothelium, mesangial, proximal and distal convoluted tubules). Furthermore, disappearance of basal infoldings and fragmentation of mitochondria in both proximal and distal convoluted tubule cells were obviously seen (Fig.4g&h).

Although various aspects of immune response and histopathology have been investigated after renal infection with *E. coli* [40], but we used a rat model nephritis that mimics severe human kidney infection to characterize the degree and nature of infection after simultaneous localization of *E. coli* and hyperlipidemia through ultrastructural study of kidney tissue. In this study, the electron microscopic results for the fourth group showed increased and massive nuclear changes in the form of pyknosis, chromat in margination and karyolysis. Most of the renal corpuscles and renal tubules were severely affected than group II&III (Fig.IVa-d). Increased lysosomes of proximal and distal convoluted tubular cells were more frequently increased than GII and GIII (Fig.IV e,i&g). Sever degeneration as well as wooly focal densities in the mitochondria were observed in distal convoluted tubules (considered to be evidence of irreversible cell injury) [41]. (Fig.IVg). Besides, complete loss of basal infoldings and thickened basal lamina that were more pronounced in distal convoluted tubules of GIV than GII&GIII (Fig.IV h).

In the effect of *E.coli* infection together with hyperlipidemia in animal model, a situation common in humans as a result of microbial agents and some other inflammatory diseases, we demonstrated from previous study that feeding high fat diet to rats injected with *E.Coli* led to a decline in renal excretory function and to the development of proteinuria[22]. These changes correlated with an increase in mesangial matrix and cellularity and tubulointerstitial alterations including macrophage influx and an increased turnover of tubular epithelial cells. The mechanism by which hyperlipidemia exerts its deleterious effect on the kidney is still largely unknown. One mechanism by which hyperlipidemia may mediate renal injury could be by directly acting on resident cells in the kidney. The glomerulus and the renal tubulointerstitium may be a preferred location for lipid
deposition and interaction with resident cells because of the lack of a basement membrane separating the mesangium and the capillary stream and the presence of fenestrated epithelium lining glomerular and peritubular capillaries[42&43]. Lipids can therefore easily access these areas and accordingly inhibition of lipoprotein manufacture which is involved in the transport of triglycerides that influence local metabolism and results in accumulation of fats in the cytoplasm[43]. Mesangial cells have been shown to bind and take up native and oxidized low-density lipoprotein (LDL) cholesterol[42]. Binding of LDL and other lipoproteins to mesangial cells has been shown to influence cellular proliferation and stimulate secretion of inflammatory mediators like prostanoids, interleukin-6 (IL-6), PDGF, and TGF- β [44]. In addition, lipoproteins influence matrix generation by stimulating mesangial fibronectin and collagen IV expression in kidney disease[45]. Moreover, various animal models of hyperlipidemia-induced renal disease have demonstrated increased influx of macrophages into glomeruli[46] and into the tubulointerstitium[47&48] of affected animals. The functional importance of macrophages in mediating renal injury in hyperlipidemic states is highlighted by the author [47] who showed that depletion of macrophages resulted in an amelioration of renal function impairment and reduction of glomerulosclerosis and matrix expansion in hyperlipidemic rats with acute puromycin aminonucleoside nephrosis. On the other hand, another study confirms increased pathological changes in (E-coli and hyperlipidemic group) that could be the result of metabolites reabsorption from proximal tubules rather than systemic stimulation, since these metabolites are extensively reabsorbed in proximal convoluted tubules tubules [49]. The marked increased alteration in proximal and distal convoluted tubules in group IV of the current study suggested an additive pathological effect that aggravate injury in kidney tissue.

V. Conclusion

In summary, we describe distinctive excess renal glomerular and tubular ultrastructural injury associated with bacteremia and hyperlipidemia, which probably represents a novel and explained form of renal injury.

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Figure 1: Electron micrographs of kidney sections of control rat (group I)

a) Showing portion of glomerulus indicating different cell types: capillary endothelial cell (EN), visceral epithelial cell (VEC), and mesangial cell (MC).

(b) High magnification of glomerular capillary wall (Cp) showing the barrier between the lumen of the glomerular capillary and the urinary space of Bowman’s capsule (Us). Note: nucleus (N) of visceral epithelium or podocyte, foot processes (Fp), fenestrated endothelium (En), glomerular basement membrane (GBM) and the inset showing a high magnification of GBM (arrow).

c) Showing euchromatic nucleus (N) of proximal convoluted tubule cell. Note: numerous mitochondria (M), basement membrane (BM) and the nucleus is relatively large, mostly euchromatic with prominent nuclei and always lying at the basal portions of the cells.

d) Showing apical microvilli (MV) constituting a brush border, below which lies the endocytotic apparatus (arrow). Note: mitochondria (M) and secondary lysosome (Ly).

e) Showing euchromatic nuclei of distal convoluted tubule appear large and lying at the apical portion of the cytoplasm (N). Note: the apical microvilli (arrow head) which do not

(f) A high magnification of numerous long basilar infoldings (arrows) of distal convoluted tubule reaching the level of nucleus (N). Note, the linear arrangement of the mitochondria (M).
Figure 11: Electron micrographs of kidney sections of hyperlipidemic rat (group II)

a) Showing irregular nuclear envelope and partial margination of heterochromatin of nuclei (N) of both mesangial cell (Mc) and podocytes (Pd). Note, pyknotic nucleus of endothelial cell (arrow).

b) Showing increased peripheral migration and interposition of mesangium. Notice, mesangial cells with marked irregular nuclear envelope and marginated heterochromatin (stars) and the mesangial matrix extended from the central lobular portion of the tuft into the peripheral capillary wall (arrows).

c) Showing limited foot processes effacement of podocytes (arrow). Note, extracellular matrix (star); pyknotic nucleus (N1) of endothelial cell (En); autolytic nucleus (N2) of podocyte.

d) Showing vacuolated cytoplasm of proximal convoluted tubule cells (arrow). Note, disintegration and fragmentation of mitochondria (thick arrow); large lipid droplets (stars).

e) Showing the cytoplasm of proximal convoluted tubule cell appeared with vacuoles of different sizes (V). Note, increased hyaline cast (arrows); thickened basal lamina (circle); irregular nuclear envelope of nucleus (N); smudgy homogenous deposits in peritubular space (stars).
f) Showing thickened basal lamina of parietal epithelium (Bm). Note fragmented orthodox mitochondria (stars); pyknotic nucleus (N); the apical microvilli still intact (arrow).
Figure I I I : Eletron micrographs of kidney sections of E-Coli treated rat (group I I I )

a) Showing loss or effacement of food processes. Note: dilution and lysis of nucleoplasm of nuclei of podocytes (Pd) or visceral epithelium; margination of heterochromatin in the nuclei of both endothelial cells (En) and mesangial cells (Mc); urinary space (Us); parietal epithelium (Pe).

b) Showing increased peripheral migration and interposition of mesangial cells (Mc) and expanded mesangial matrix (arrow).

c) Showing urinary space (Us) and lumen of capillary occupied by dense infiltrations (star); continuous granular density in glomerular basement membrane (arrow).

d) Showing accumulation of infiltrations in the lumen of capillaries (stars); margination of heterochromatin in nuclei of both endothelial cell (N1) and mesangial cell (N2); thickened basal lamina of parietal epithelium (circle).

e) Showing eosinophil (arrow); endothelial cell (En); mesangial cell (Mc); red blood cells (RBC).

f) Showing widened intertubular space (stars); pyknotic nucleus of interstitial cells (arrow); irregular nuclear envelope of nucleus (N); fragmentation of mitochondria (white arrow).

g) Showing pyknotic nuclei (N1) of distal convoluted tubule cells; nucleus (N2) have moved from base to apex.

h) Showing numerous lysosomes (arrows) and a considerable amount of hyaline cast filled the lumen (star).

i) Showing disappearance of basal infoldings in distal convoluted tubule cells (stars); fragmented mitochondria (black arrows); diluted mitochondria (white arrows); the brush border still intact (Mv).
Figure IV: Electron micrographs of kidney sections of Hyperlipidemia and E-Coli treated rat (group IV)

a) Showing glomerulus with incomplete effacement of foot processes of podocytes (arrows). Notice: thickened glomerular basement membrane (circle); pyknotic endothelial nucleus (N); closed endothelial capillaries with infiltration (stars).

b) Showing margination of heterochromatin of endothelial nucleus (N) and irregularity of nuclear envelope. Notice: marked thickened glomerular basement membrane (circle); intensive smudgy deposits in luminal capillary (stars).

c) Showing hypercellularity and margination of heterochromatin of podocytes (stars). Notice: complete effacement of food processes (arrows).

d) Enlarged portion of fig.c showing loss of normal fenestration of endothelial cells (arrow) in addition to loss of differentiation of the three layers of glomerular basement membrane (thick arrow). Notice: nuclei (N) of endothelium.

e) Showing proximal convoluted tubules with numerous lysosomes (short arrows). Notice: nuclei of different sizes and shapes showing different stages between margination, and pyknosis (arrow).

f) Showing irregular nuclear envelope of nucleus (N). Notice: complete disintegration and fragmentation of mitochondria (M); increased pinocytotic vesicles (short arrows); thickened basal lamina (circle).

g) Showing proximal convoluted tubule cells with dilution and lysis of nuclear nucleoplasm (N); numerous lysosomes (short arrows); disrupted microvilli (Mv) at the lumen (L).

h) Showing distal convoluted tubule cells with swollen cytoplasm. Notice: pyknotic nuclei (N) (stars); orthodox mitochondria (M) were limited to the base only; closed lumen with complete distortion to the apical microvilli (arrow); disappearance of basal infoldings (short arrow); marked thickening of basal lamina (circle).