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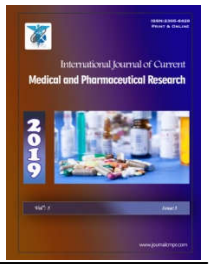

ELECTRON MICROSCOPIC STUDYIN FULL TERM HUMAN ABORTED FETUS WITH FUTURE IMPLICATIONS OF CADAVERIC FETAL KIDNEY TRANSPLANTATIONINEND STAGE RENAL DISEASE PATIENTS

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ABSTRACT

The three dimensional fine structure of human glomeruli taken from full term aborted human fetus was studied under the scanning electron microscope. The podocytes and their cytoplasmic processes with their elaborate ramification on the capillary wall were clearly demarcated in the glomerulus of the tissues which were paraffin embedded and then cleared in xylol and fixed byperfusion of glutaraldehyde and paraformaldehyde, and in the glomerulus of the biopsy specimen fixed by immersion in the osmium tetraoxide secondary fixative. The human podocytes were characterized by marked irregularity in the ramification pattern of their cytoplasmic processes, as compared with those of the rat and the rabbit.The interdigitation of the terminal processes was always noticed between those of different cells. In this study attempt was made to apply scanning electron microscopy to the human kidney specimens of full term aborted human foetuses and to clarify the three-dimensional fine structure of the human glomeruli, especially of the podocytes, structure of glomerular and the cytoplasmic processes in view of the glomerular changes in End Stage Renal Disease and Obstructive Uropathy. The diseases of Basement Membrane like Alport's Syndrome and Good Pasture Syndrome can also be elicitated by this study.

INTRODUCTION

The use of isolated glomeruli was first described byArakawa in 1971[1]and since then these have been employed by several workers[2,3] for studying human glomerular diseases with the scanning electron microscope. As Ng WL et al[4] pointed out, however the use of scanning electron microscopy alone is of little diagnostic value for such study. This is because the surface changes of various disease states are not well understood and documented. Scanning Electron Microscopy was considered best for the stereoscopic structural study of the kidney surface by some researchers in lower vertebrates till date [15]. Based on transmission electron microscope findings of tissue sections of kidney by earlier researchers, the current view of the three-dimensional structure of the glomerulus, particularly of its podocytes, has some topographic relation with adjacent podocytes, the cellular origin and pattern of cytoplasmic processes [6]. However, only the studies of the glomerulus in animals, including rats and rabbits, in normal and some pathologic situations have been reported until now [7]. Without any doubt, it will be of great advantage if this methodology in human aborted full-term foetuses is viewed as important in clinical nephrology [5]. An attempt was made in this study to apply scanning electron microscopy to the human kidney specimens of full term aborted human foetuses and to clarify the three-dimensional fine structure of the human glomeruli, especially of the podocytes in view of the glomerular changes in End Stage Renal Disease. The diseases of Basement Membrane like Alport's Syndrome and Good Pasture Syndrome can also be elicitated by this study.[8,9]

Aims and Objectives

The present study aims to study histogenesis and development of human kidney in prenatal period to observe ultrastructure of kidney in human full term aborted fetus and its future implications in cadaveric fetal kidney transplantations in End Stage Renal Disease patients.

MATERIALS & METHODS

The tissue material from kidney of a 37-week-old foetus which did not show any evidence of morphological abnormality was collected from Department of Gynaecology and Obstetrics in a tertiary medical college in Bhubaneswar after spontaneous miscarriage after approval of the Institutional Ethical Committee of Human Research. This was collected after written informed consent of the legal guardian accorded with institutional guidelines. Foetus was immediately fixed in 10% formalin for 1-2 hrs. Kidney was dissected by dissecting microscope, fixed in 10% formalin for 24 hrs after cutting it in 2mm size. After fixation by formalin, it was then processed for

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paraffin sections and then they were embedded. The embedded tissue was cleared in 4 changes of xylene 30 min each at 37 °C. Rehydrated with 4 changes of 100% ethanol - 10 min each, 70% ethanol - 5 min, 30% ethanol - 5 min. The tissue was washed with three changes of 0.1M Phosphate Buffer 15 min each at 4°C and then the section was flooded with 1% osmium tetroxide (OsO4) to post-fix for 2 hours at 4°C. Then it was washed with three changes of 0.1M Phosphate Buffer 15 min each at 4°C. Then it was dehydrated with three changes of acetone 15 min each and then 2 changes in dry acetone 15 min each. Then the sample was then transferred to liquid CO2 in a chamber that is cooled and put under pressure. When acetone had been completely removed and the tissue was impregnated with liquid CO2, the chamber were warmed up to critical point 31.5°C where the density of drying medium was same in both liquid and gas phase at 1100p.s.i. (Critical Point Drying). After drying the specimens they were then mounted on aluminium stubs with conductive paint (silver or copper paint) with the area of interest exposed towards the surface and then coating was done with gold and argon gas was flooded in chamber for 1 min, ultimately a uniform thin layer was deposited on the specimen (Sputter Coating).After metal coating the specimen was ready for observation under Scanning Electron Microscope.[13,16]

Observations

Both sectioned and intact glomeruli were seen. Sectioned glomeruli were found mostly on the smooth sectioned surface which appeared clean and devoid of artefacts. The sectioned glomeruli showed intact internal architecture with recognisable epithelial cells, capillaries and mesangial areas. Internal surface morphology of Bowman's capsule, glomerular vasculature and mesangial cell proliferation was revealed. Intact glomeruli with Bowman's capsules were also seen. The inner surface of the remaining capsule was covered by the flattened epithelium. A finely reticular tissue presumably corresponding to the basement membrane was partly exposed on the outer surface. The glomerulus revealed winding blood capillary loops, which were covered by podocytes. The cell body of a podocyte, containing a nucleus, was located mostly inside, but sometimes outside, of the curve of capillary loops. It was rather thick and round, but occasionally flat. Several cytoplasmic broad plate-like processes (primary processes), extended in various directions from the cell body and narrow band-like processes widening occasionally in their peripheral portions. Irregular secondary processes occasionally issued from some of the primary processes and also ended in irregularly expanded plates. Tertiary processes, which were even more irregular in size and shape, were rarely seen to emerge from the secondary processes. From the primary, secondary and tertiary processes, but not from the cell body itself, many short and thin terminal processes emerged to lie on the capillary wall which corresponded to the foot processes. They were very irregular in length, width and shape; clubbed, twisted and even branched forms occurred. They issued at random angles and the regular fern-leaf pattern of the terminal processes.

Some of the capillary loops were not well expanded by perfusion fixation. Even the cell body itself often appeared folded, with irregular elevations and grooves on those collapsed capillary walls. The surface of the cell body of the podocyte, under high magnification, was irregularly granular and uneven in appearance. Thin sting-like micro projections of the cytoplasm were frequently seen on the cell bodies and cytoplasmic processes. In the peripheral, attenuated portion of the cell body, and in some plate-like processes, there were occasionally tiny pores, through which micro projections of underlining processes could be seen and some even came out to the cell surface.

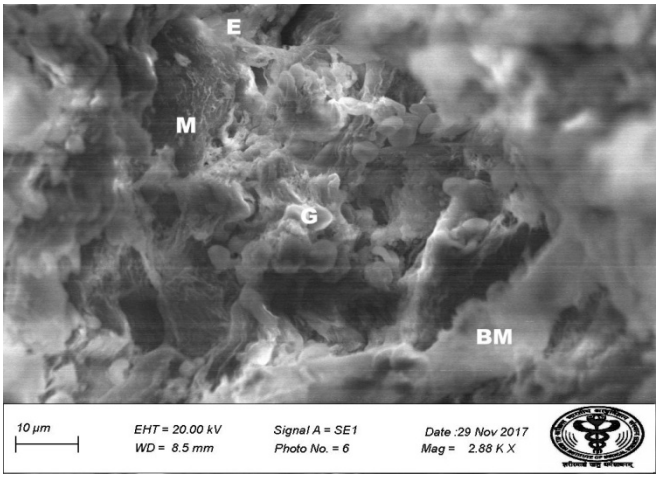

Fig 1 E-Epithelium, G-Glomerulus, M-Mesangial Cells, BM-Basement Membrane

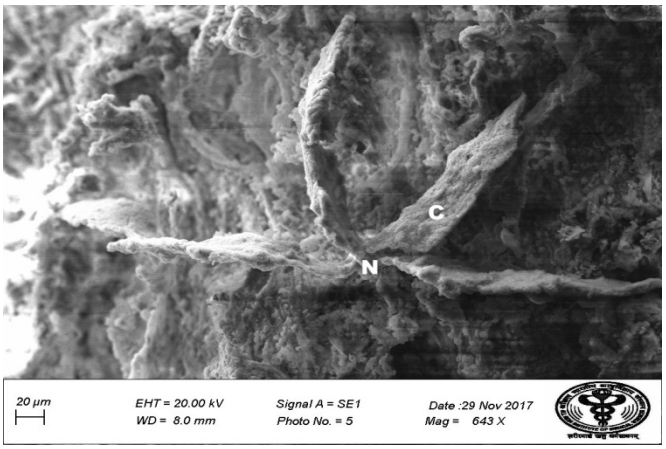

Fig 2 N-Nuclear Portion of Podocytes, C-Cytoplasmic Processes

DICUSSION

Scanning electron microscopy of the podocytes of human glomeruli, which was first attempted in this study, revealed essentially the same structure as in rats and rabbits. The most striking difference between human glomeruli and those of other animals was the form of podocyte processes. The primary, secondary, tertiary and even terminal processes were markedly irregular in shape, length and direction, compared with the uniform pattern of the processes in the rat and with the less uniform one of the rabbit. Pease [10]and Yamada,[11]in their studies showed that the adjacent terminal processes came always from different cells. Buss and Kronert [12]who performed the first scanning electron microscopy of the glomerulus using the rat, described neighbouring processes as coming partly from the same cell. Our findings correlated well with the studies done by earlier researchers nationwide and worldwide.

CONCLUSION

The detailed ultra structural study can help in the diagnosis of Basement Membrane diseases like Alport syndrome and Good pasture Syndrome [14]. Due to abundance of capillary loops and vasculogenesis, the fetal kidney transplantation may be

possible in future for End Stage Renal Diseases thus minimising the chances of Graft Rejection.This study should be correlated with Immunoelectron Microscopy and Transmission Emission Microscopy which will very well give the detailed architecture of the fetal histogenesis of kidney and the antigens expressed on the surface of kidney which may enrich the therapeutic approach to renal cell carcinoma

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