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Scanning electron microscopic studies on the postnatal alterations in the renal corpuscle of Indian domestic pig *(Sus scrofa domesticus)*#

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Abstract

Ultrastructural studies were conducted on the renal corpuscle (RC) of postnatal porcine kidney. The study was carried out in the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar on 18 pairs of fresh kidney specimens collected from slaughter houses in and around Hyderabad. The specimens were divided into three groups based on the age of the animals. SEM features of Group I kidneys (0-4 weeks) revealed renal cortex with immature nephrons near the capsule and mature nephrons close to renal medulla. The surface of mature glomeruli was covered by podocytes which consisted of a cell body with central nucleus visible as a prominent bulge. The podocytes had primary cytoplasmic processes which branched into secondary and tertiary processes and formed fine inter-digitating slits with adjacent podocyte processes. Tri-lobular appearance of glomeruli was also observed in Group III (>10 weeks).

Keywords: *Renal corpuscle, glomerulus, filtration barrier, podocytes, glomerular basement membrane*

Mammalian kidneys remove nitrogenous and other waste metabolic products and also help maintain blood homeostasis (Potter, 1972). In most mammals, nephrogenesis is incomplete at birth resulting in a relatively lower efficiency of kidney in newborn animals than in adults (Loggie *et al.,* 1975). Nephrogenesis gets completed prior to birth in humans, monkeys, mice, sheep and guinea pigs, while after birth in rats, dogs, and pigs (Zoetis and Hurtt, 2003). Postnatal growth and functional changes are marked and are adapted to extra-uterine life and also progress to adult renal function. Juxta glomerular (JG) apparatus consists of modified smooth muscle cells called 'juxta-glomerular cells' located in the wall of afferent arterioles, macula densa of the distal convoluted tubules and extraglomerular mesangial cells. Few JG cells may be present in the wall of efferent arterioles (Kierszenbaum, 2002). Evidence gathered about the morphological and functional similarity between the human and the porcine kidney is abundant, resulting in the use of this species for experimental models and for therapeutic and surgical applications (Gomez *et al*., 1999). However, studies on the postnatal development of porcine kidney is limited. Hence, the present study was aimed to obtain anatomical information on the postnatal development of kidney in pig with particular reference to age related anatomical changes in renal corpuscle.

#Part of PhD thesis submitted to P. V. Narasimha Rao Telangana Veterinary University, Hyderabad, Telangana

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Materials and methods

The present investigation was carried out in the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad, India. 18 pairs of fresh kidney specimens were collected from three age groups *viz.*, Group I (0-4 weeks), Group II (4-8 weeks) and Group III (>10 weeks) of pigs at regular weekly intervals. Unfixed fresh kidney tissue specimens of one cubic mm size were cut and fixed in 2.5% EM grade glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.2) and kept for 24 hrs at 4ºC as per the standard procedure of Ruska Labs, College of Veterinary Science, Hyderabad.

Scanning Electron Microscopy Procedure

For SEM studies, fixed tissue samples (in EM grade 2.5% glutaraldehyde solution) were post fixed in 1% aqueous Osmium tetroxide in same buffer for 4 hrs. Later they were dehydrated in series of graded alcohols and dried to critical point drying with CPD unit (EMS 850) / vacuum desiccation for 35-45 minutes for complete drying of specimens. Dried samples were mounted over stubs with double-sided carbon conductivity tape and coated by a thin layer of heavy metal (gold) using an automated sputter coater (model –JEOL, JFC-1600) for 3 minutes and scanned under Scanning Electron Microscope (Model: JOEL-JSM 5600, JAPAN) at required magnifications as per the standard procedure of RUSKA labs, Department of Veterinary Pathology, College of Veterinary Sciences, Hyderabad, India. SEM details of the entire Bowman's capsule in meridional section and glomerular tuft were studied and recorded. Microphotographs at desired magnification were taken and recorded.

The present investigation and its experimental design were approved by the Institutional Animal Ethics Committee vide No.39/24/ IAEC-Pig / C.V.Sc./ Hyd. Dt: 12.06.2021.

Results and discussion

The kidneys of Group I showed renal cortex with renal corpuscles, glomeruli, proximal and collecting tubules at multiple developmental stages. Immature nephrons were observed on the outer surface of the renal cortex nearer to the capsule, whereas mature nephrons were found close to the renal medulla (Fig. 1). The outer cortex showed different stages and shapes of nephron development (Figs. 2 and 3). The developmental stages of the nephron like mesenchymal condensates (Fig. 4), renal vesicles (Figs. 5 and 6), and comma and S-shaped bodies (Fig. 7) were visualized. Glomeruli in capillary loop stage showed bulbous projections (Fig. 8). Mature glomerulus showed podocytes with primary processes from which several smaller processes formed an intricate fine inter-digitating structure (Fig. 9). These findings collaborate with the description of renal cortex by Kazimierczak (1980) and Fayez *et.al*. (2014) in rats and rabbits respectively through SEM studies.

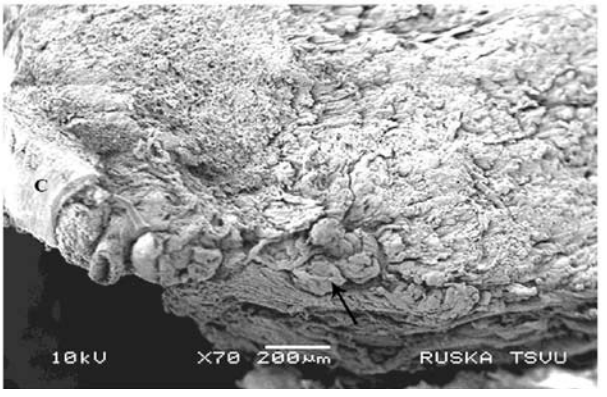

Fig. 1. Scanning electron micrograph of 7 PND cortex (Group I) showing the renal cortex with glomeruli (➔) in different stages of development. C-Capsule SEM 70x

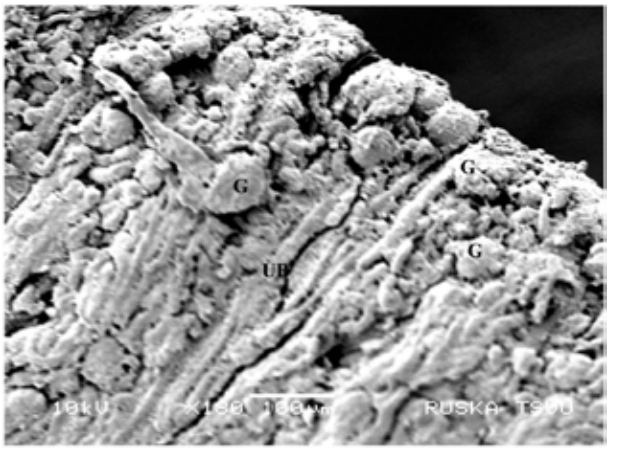

Fig. 2. Scanning electron micrograph of 7 PND cortex (Group I) showing the cortex with developing glomeruli(G) around ureteric bud (UB). SEM 180x

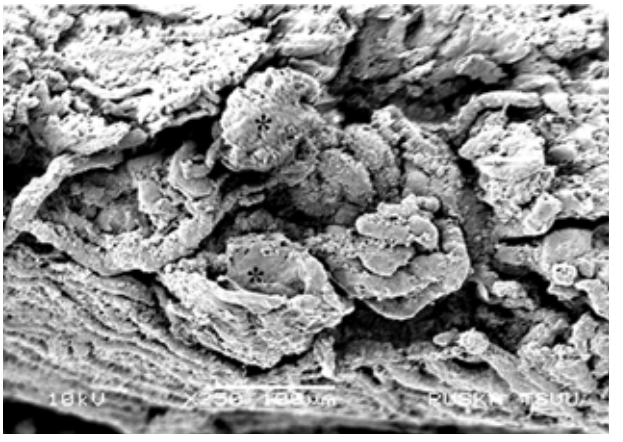

Fig. 3. Scanning electron micrograph of 7 PND cortex (Group I) showing renal cortex with glomeruli (*) in different stages of development. SEM 250x

The kidneys of Group II and Group III showed fully developed renal corpuscles in all the zones namely subcapsular, mid-cortical and juxtamedullary zones. Renal corpuscles in all the groups were mostly

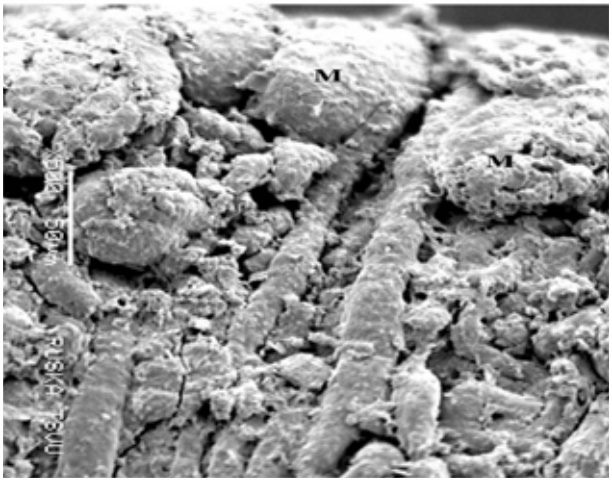

Fig. 4. Scanning electron micrograph of 7 PND cortex (Group I) showing the mesenchymal condensates (M). SEM 2000 x

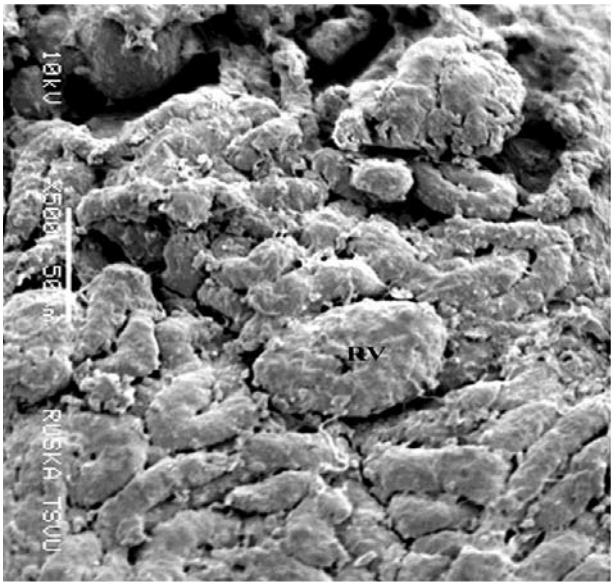

Fig. 5. Scanning electron micrograph of 7 PND cortex (Group I) showing the renal cortex with the forming renal vesicle (RV). SEM 500X

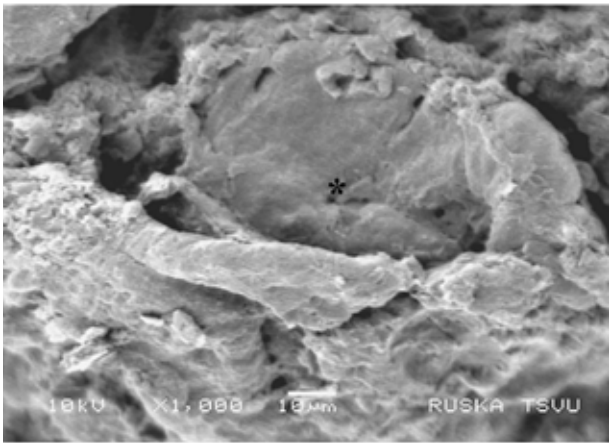

Fig. 6. Scanning electron micrograph of 7 PND cortex (Group I) showing the vesiclular stage of glomerulus (*). SEM 1000 x

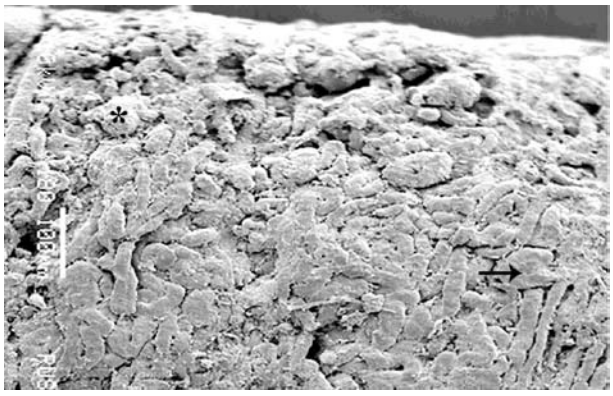

Fig. 7. Scanning electron micrograph of 7 PND cortex (Group I) showing the comma (➔) and S shaped (*) stages of glomerulus. SEM 180x

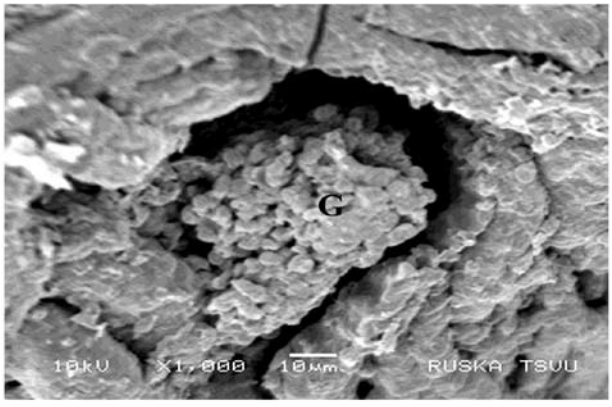

Fig. 8. Scanning electron micrograph of 7 PND renal cortex (Group I) showing glomeruli (G) in capillary loop stage. SEM 1000X

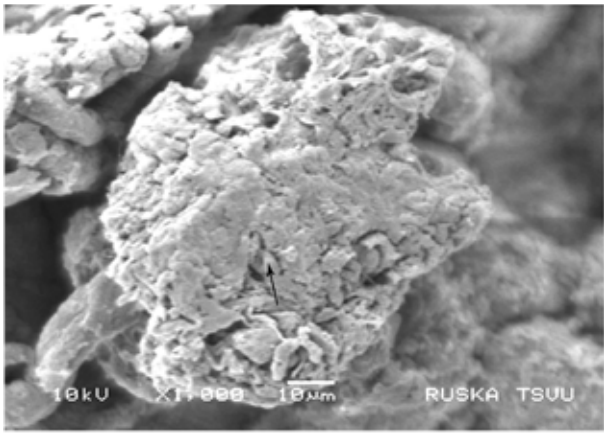

Fig. 9. Scanning electron micrograph of 7 PND cortex (Group I) showing Glomeruli with primary processes (➔) in juxtamedullary region. SEM 1000 x

spherical with a clearly outlined parietal epithelium of the Bowman's capsule (BC). Glomerulus was located inside the Bowman's capsule (BC). The urinary space was a narrow space between the visceral and the parietal layer of bowman's capsule (Fig. 10). The parietal epithelium was lined by simple squamous cells. Bridging cytoplasmic processes from parietal cells extended to the glomerulus (Fig. 10). The surface of glomerulus was rough and

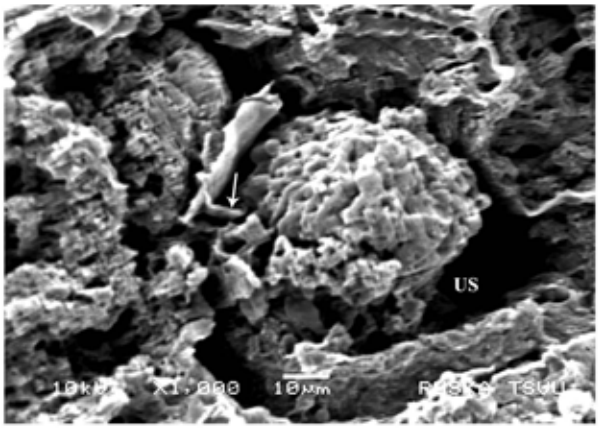

Fig. 10. Scanning electron micrograph of 63 PND cortex (Group II) showing the midcortical renal corpuscle. Urinary space (US), Bridging process- ➔ SEM 1000 x

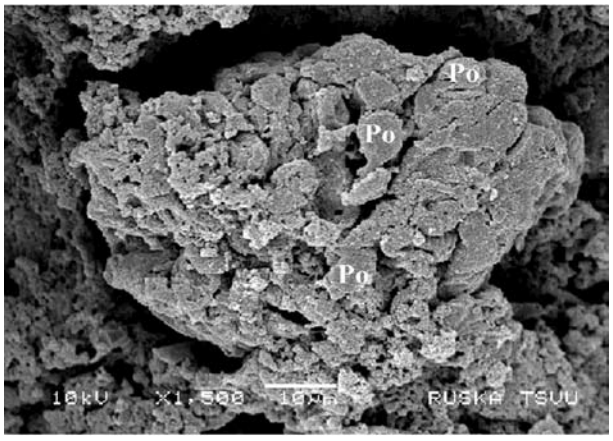

Fig. 11. Scanning electron micrograph of 152PND cortex (Group III) showing the juxtamedullary glomerulus with podocytes (Po) and their processes (➔). SEM 1500 x

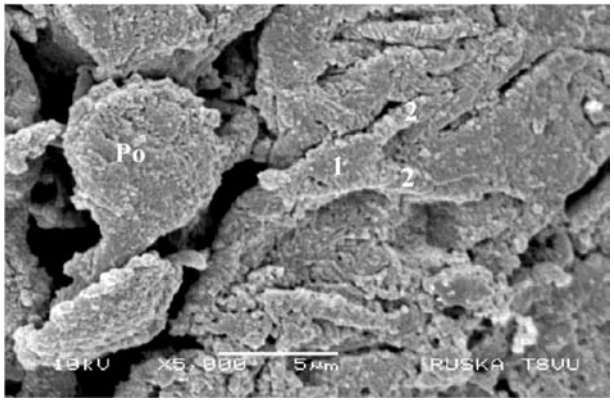

Fig. 12. Scanning electron micrograph of 152 PND cortex (Group III) showing the juxtamedullary renal corpuscle with podocytes and their interdigitating processes (➔). 1. Primary processes 2. Secondary processes SEM 5000X

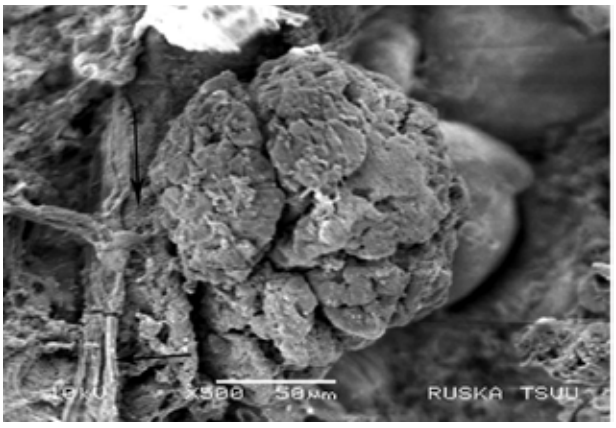

Fig. 13. Scanning electron micrograph of 152 PND cortex (Group III) showing the juxtamedullary multilobed glomerulus with arterioles (➔). SEM 500 x

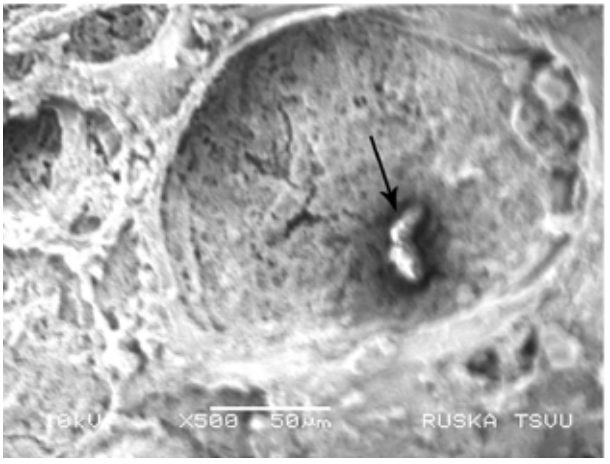

Fig. 14. Scanning electron micrograph of 152PNDcortex (Group III) showing parietal layer of Bowman's capsule (*) and urinary pole (➔) SEM 500 x

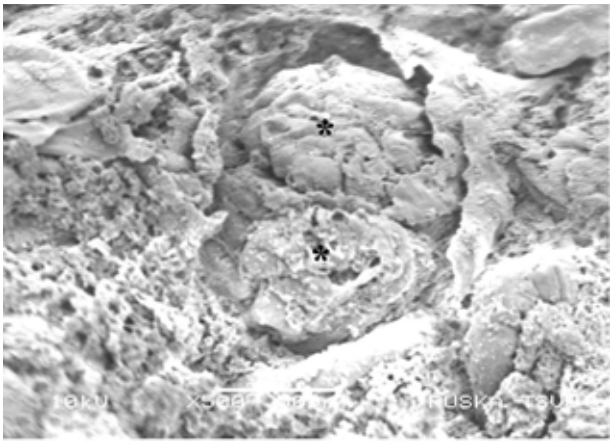

Fig. 15. Scanning electron micrograph of 152 PND cortex (Group III) showing the juxtamedullary bilobed glomerulus (*). SEM 500x

covered by finely modified cells of visceral epithelium. These cells called podocytes consisted of a cell body with central nucleus visible as a prominent bulge. These findings are in concurrence with the reports of Andrews and Porter (1974) and Webber and Lee (1974) on RCs in albino rats and humans, respectively. They observed a remarkably thin parietal epithelium of BC, which exhibited many indentations enveloping spherical glomeruli. Primary cytoplasmic processes extended in a tentacle-like fashion encircling the capillaries in the glomerulus. Primary processes gave origin to secondary and tertiary processes which formed fine inter-digitating structure with adjacent podocyte processes forming filtration slits

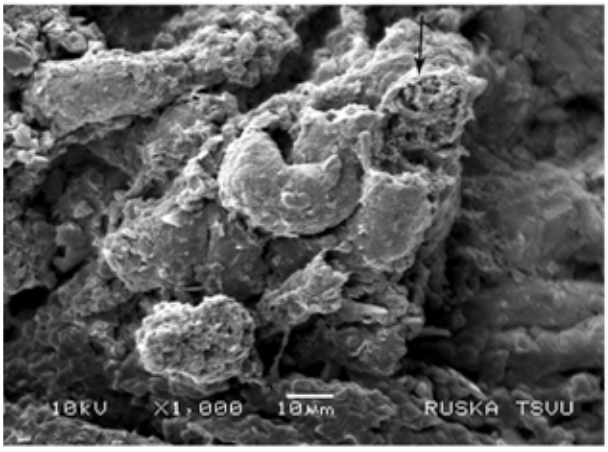

Fig. 16. Scanning electron micrograph of 152 PND cortex (Group III) showing the proximal convoluted tubule (➔). SEM 1000 x

(Figs. 11 and 12). These reports also are in parity with the observations of Gibson *et.al.* (1992) in humans and Pavestandt *et.al*. (2003) in rats who cited that podocytes gave rise to long primary processes, which were affixed by numerous foot processes to the neighboring capillaries. The authors stated that foot processes of neighboring podocytes regularly inter digitated with filtration slits.

Afferent and efferent arteriole were observed at the vascular pole (Fig. 13). Urinary pole was observed on the opposite end (Fig. 14). Lobular appearance of glomerulus was evident in Group III (Fig. 15). Lobules were formed by capillary loops which were enveloped by visceral epithelium of BC. In adjunct with the present findings, Reddy (2018) reported that AA entered and formed two to three capillary loops which rendered a tri-lobular spherical or elliptical appearance to the glomerulus in goats and pigs. PCT cut sections were appreciated external to the BC (Fig. 16), which showed narrow lumen with fine ciliated process emanating from lining epithelium. These observations are in parity with reports of Simsek *et.al.* (2009) in rats.

Conclusion

The SEM features of Group I kidneys revealed renal cortex with RC, glomeruli, PCT, DCT and collecting tubules at multiple developmental stages. Immature nephrons were observed on the outer surface of the renal cortex, while mature nephrons were found close to the renal medulla. The outer cortex showed different stages and shapes of nephron development while mature glomeruli were observed in the deeper zones as well as other groups.

Acknowledgements

We would place on record my gratitude towards P.V. Narsimha Rao Telangana Veterinary University, Hyderabad, for providing all the necessities during my investigation to prosecute my doctorate studies.

Conflict of interest

There is no conflict of interest among the authors.

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