Expression analysis of leptin in nephrogenesis and renal carcinogenesis

Priyanka Parmesh[1], Roshni Sadashiv[2], U.S. Dinesh[3], Anil Bargale[4]

[1] *Dept. of Pathology, SDM University, SDM College of Medical Sciences & Hospital, Dharwad, India*
[2] *Dept. of Anatomy, SDM University, SDM College of Medical Sciences & Hospital, Dharwad, India*
[3] *Dept. of Pathology, SDM College of Medical Sciences& Hospital, Dharwad, India*
[4] *Dept. of Biochemistry, SDM College of Medical Sciences& Hospital, Dharwad, India*

SUMMARY

The complex phenomenon of renal development involves several signaling molecules. Any alteration in the developmental process could largely influence the organogenesis, thus predisposing to several adulthood diseases. Leptin is a pleiotropic gene secreted by adipose cells. However, the purview of its actions is much beyond merely the extent of human adipose reserve. It regulates several cellular mechanisms such as cell proliferation, inflammation, vasculogenesis, and the production of collagen fibres. Further leptins are thought to play a putative role in embryogenesis and tumorigenesis. The aim of thIS study was to investigate the localization of the protein in fetal and cancer kidney in an attempt to understand the role of the protein in fetal kidney development and in renal cancer.

Leptin expression was evaluated by subjecting tissue sections from paraffin-embedded blocks of renal tissues (fetal, adult and cancer) to immunohistochemistry staining. The tissues were scored based on the staining pattern and percentage of immunoreactive cells. The tissues were subjected to haematoxylin and eosin stain before performing immunohistochemistry. The images were analyzed and photographed. Mild staining for leptin was observed in the tubules of fetal and adult kidneys. Mild to moderate staining was seen in membranes of renal cell carcinoma tissues. It appears such that leptin may not be a key factor or rather a temporary factor in the developmental process of the kidney. The low levels of leptin in normal adult renal tissues may be physiologically significant. The role of leptin in the renal cell carcinoma progression is sceptible.

Keywords: Leptin – Renal cell carcinoma – Kidney – Nephrogenesis – Immunohistochemistry

INTRODUCTION

Leptin, a16kDa hormone is produced in the intrauterine life. Its varying concentration in the systemic tissues has been identified in the foetal and neonatal life in mice (Reynolds and Vickers,

Corresponding author:
Dr. Roshni Sadashiv. Dept. of Anatomy, SDM University, SDM College of Medical Sciences & Hospital, Dharwad, Karnataka, India. Phone: +91 9480242975; Fax: 0836 2461651. E-mail: drroshnisadashiv@gmail.com

Part of this work was presented at the meeting: 'International Conference on Biomedical and Clinical Research', organized by SHRI Dharmasthala Manjunatheshwara University in Dharwad, India on November 21-22, 2022.

Submitted: November 2, 2023. **Accepted:** December 20, 2023

https://doi.org/10.52083/MYAZ1507

2019) Physiologically the hormone is greatly associated with appetite and body mass index. However, the purview of its actions is much beyond merely the extent of human adipose reserve. It is thought to be involved in the biological processes of cell proliferation, angiogenesis, and reproduction. Leptin plays a role in implantation and is also produced by the trophoblastic cells indicated by its presence in the placenta, amniotic fluid, and umbilical cord (Biesianda et al., 2016).

Though leptin is primarily secreted by the adipocytes of white adipose tissue, its localization and function in other gestational tissues still need to be elucidated (Lappas et al., 2005). Leptin receptors have been localized in the central nervous tissue, lung tissue, and several other peripheral tissues (Prince et al., 2007). The definitive human kidney begins its development in the fifth week of gestational life. It is formed by an interaction between the metanephric blastema cells and a diverticulum of the mesonephric duct, the ureteric bud[5]. Several genes have been identified by experimental studies on cell lines and animal models, which are responsible for the cascade of events during nephrogenesis. Any mutations in the signaling molecules or disruptions in the epigenetic pathway could largely dispose the adult kidney to diseases such as cancer (Patel and Dressler, 2013). Our previous work on foetal renal tissues has highlighted certain less-known genes in nephrogenesis and renal cancer (Sadashiv et al., 2019).

Owing to the different cell types in the human kidney, the current study aimed to localize the expression status of leptin in foetal, adult, and cancer kidneys to check if leptin may have a role in kidney development and cancer.

MATERIALS AND METHODS

Representative samples of autopsied foetal kidney n=30, adult kidney (cadaveric) n=30, and renal cell carcinoma (biopsy tissues) n=30, consisted of paraffin-embedded tissue blocks collected from the Department of Pathology after approval from the institutional review board. Prior to performing immunohistochemistry, the tissues were subjected to conventional H&E staining. The histogenesis of developing foetal kidneys at different gestational ages and the diagnostic confirmation of the renal cancer tissues were done by clinical pathologists.

Four µm thick sections were taken on positively charged slides. Overnight incubation at 37ºC and deparaffinization with repeated washes of xylene (10 mins each) was followed by rehydration of tissues with graded alcohol. The slides were then washed with running water (10 mins) and distilled water (5 mins), following which antigen retrieval with citrate buffer at 95ºC was done and cooled at room temperature.

Peroxidase block was carried out for 10-15 mins and washed in phosphate buffer solution (PBS) for 5 mins and incubated with primary antibody (anti-leptin rabbit polyclonal antibody (in 1: 250 dilutions with reagent –antibody diluents as per manufacturer's instructions, {Wuhan Fine Biotech Co. Ltd, Wuhan, China} for 45 min. Subsequently, tissues were incubated with poly excel target binder for 15 to 20 mins and washed with PBS for 5 mins. Incubation was done with H.R.P for 15 to 20 mins, washed with PBS for 5 mins, and developed with DAB (diaminobenzidine) chromogen for 5-8 mins. Sections were counterstained with haematoxylin after washing with running water. Every staining run contained a slide treated with tris buffer in place of the leptin antibody as a negative control. Sections of benign breast lesion with ductal epithelial cells displaying cytoplasmic positivity was used as positive controls.

Immunoreactivity score (IRS)-classification scoring systems were used as shown in Table 1 (Fuhrman et al., 1982). For statistical purposes, the results of IRS scores were divided as follows:

Negative, mild staining = negative expression of leptin

Moderate, strong staining = positive expression of leptin

Table 1. Leptin expression in PCT cells and RCC.

Group	0	+1	+2	+3	P-value
Fetal Kidney (n=30)	27	3	—	—	
Adult Kidney (n=30)	0	22	8	—	<0.0001
RCC (n=30)	0	10	15	5	

IHC scoring for leptin antibody (0=Absent, +1=Mild, +2= Moderate, +3=Strong)

RESULTS

Expression of leptin in fetal renal tissues: as depicted in Fig. 1a and 1b, the histological sections of fetal kidney at early gestational ages showed cortex with precursors of mature glomeruli such as the renal vesicle (rv), comma, S-shaped bodies in the nephrogenic zone, and branching ureteric bud (ub). The deeper cortex demonstrated sections of developing proximal convoluted tubules (PCT), distal convoluted tubules (DCT), and thick ascending limbs of loops of Henle (LOH) (Fig. 2a, 2b). The medulla presented with developing collecting duct and blood vessels. Beyond 36 weeks of gestation, definitive glomeruli, and mature forms of the renal tubules could be demonstrated. Immunohistochemistry staining with anti-leptin antibody showed mild staining (+1) n=3 to the absence of immunoreactive cells in the PCTs' of fetal kidney from 14 weeks to beyond 36 weeks of gestation (p=<0.0001) (Fig. 2c, 2d). However, all the other cellular components of the kidneys from 14 weeks to 39 weeks were immunonegative (Figs. 1b, 2c, 2d).

Expression of leptin in adult renal tissue: adult PCT were identified by the presence of highly eosinophilic brush-bordered cuboidal epithelial cells with small lumens as shown in Figure 3a and 3b as stained with hematoxylin and eosin stain. The adult renal kidney showed mild immunopositivity in the cytoplasm of proximal convoluted tubules (+1) n=22 (Fig. 3c, 3d). The cells of the glomeruli (mesangial, podocytes, and endothelial cells), juxta glomerular apparatus, distal convoluted tubules, and loop of Henle cells were found to be immunonegative to leptin antibodies.

Expression of leptin in renal cell cancer tissue: The H and E stain showed various grades of conventional renal cell carcinoma. (Fig. 4a, 4b, 4c). The tissues were graded according to Fuhrman et al (Fuhrman et al., 1982). The conventional renal carcinoma tissues of clear cells and papillary variants showed mild to moderate cytosolic and membranous expression of leptin (+2) (n= 15), p=<0.0001) as demonstrated in Figs. 4d, 4e, 4f, 4g, 4h and 4i. Strong expression of leptin was found in fewer tissues of grade 3 renal cell carcinomatous tissues (n=5) (Fig. 4i). The proximal convoluted tubules in the normal renal cortical area adjacent to the carcinomatous tissue showed moderate staining for leptin (+2) as shown in Fig. 4d. The summary of the results is shown in Table 1.

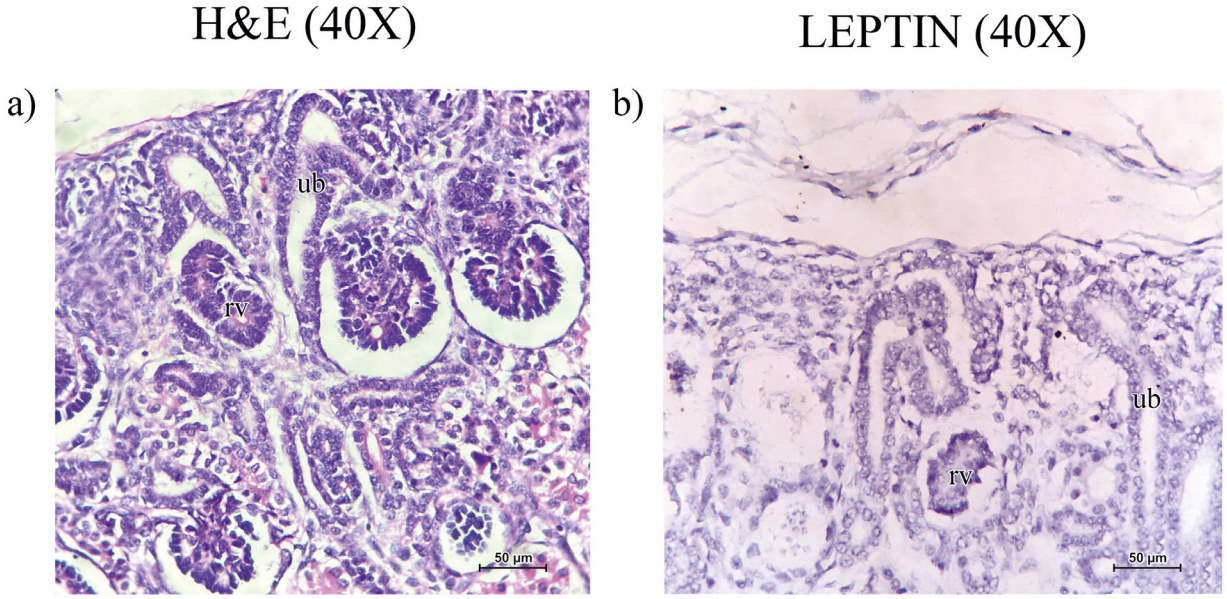

Fig. 1.- a) and **b)** show the nephrogenic zone in fetal kidney. rv, renal vesicle., ub, ureteric bud. Fig. 1b demonstrates the absence of leptin in the nephrogenic zone. Scale bars = 50 µm.

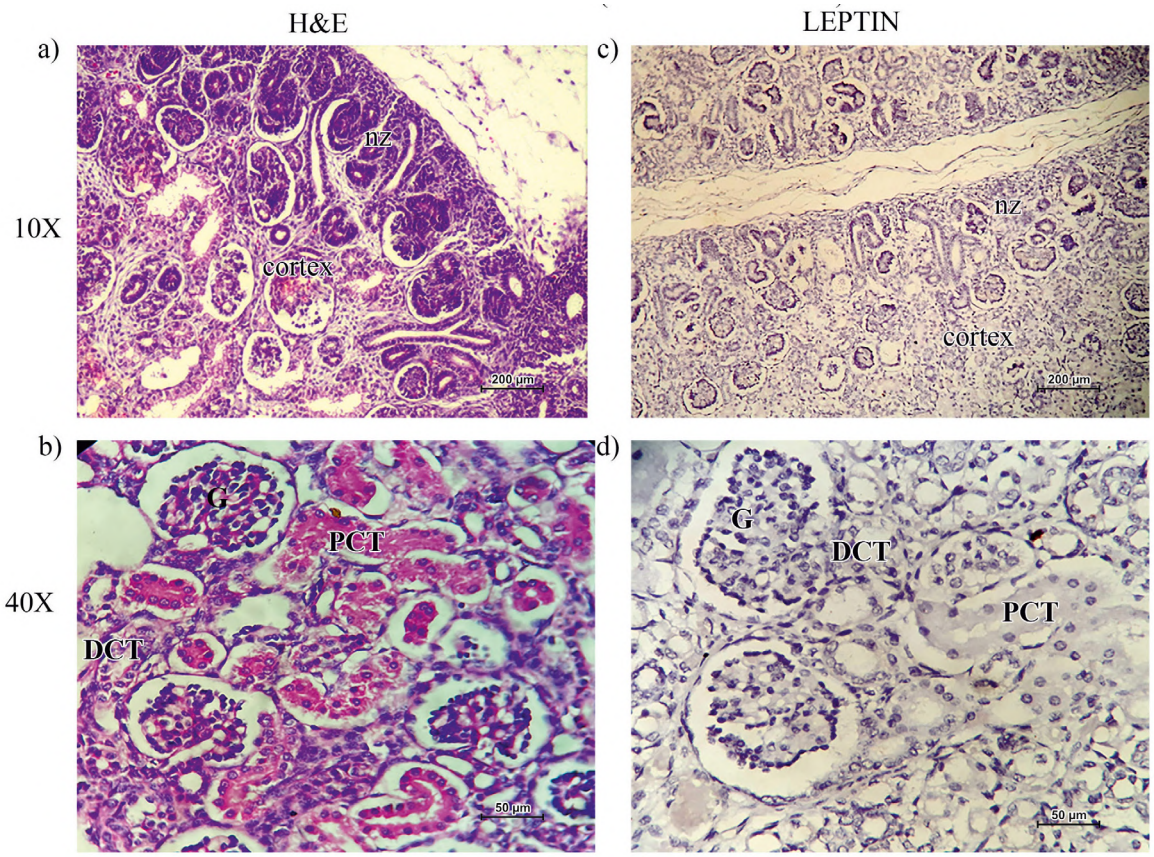

Fig. 2.- a) and **b)** demonstrate H&E sections of the fetal kidney showing the nephrogenic zone, PCT, DCT, and glomerulus (G). **c)** and **d)** demonstrate immunonegative expression of leptin in renal components. Scale bars: a, c = 200 µm; b, d = 50 µm.

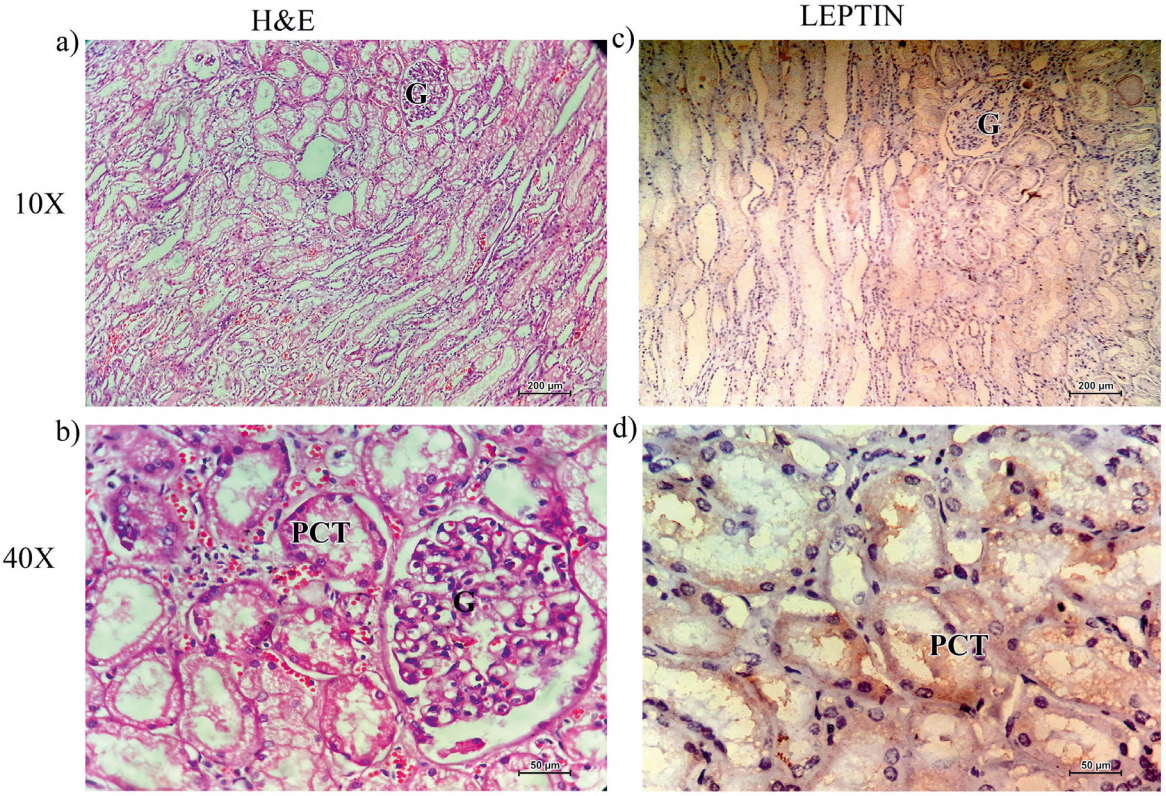

Fig. 3.- a) and **b)** show H&E sections of the adult kidney. **c)** and **d)** showing immunopositive expression of leptin in the PCTs. Scale bars: a, c = 200 µm; b, d = 50 µm.

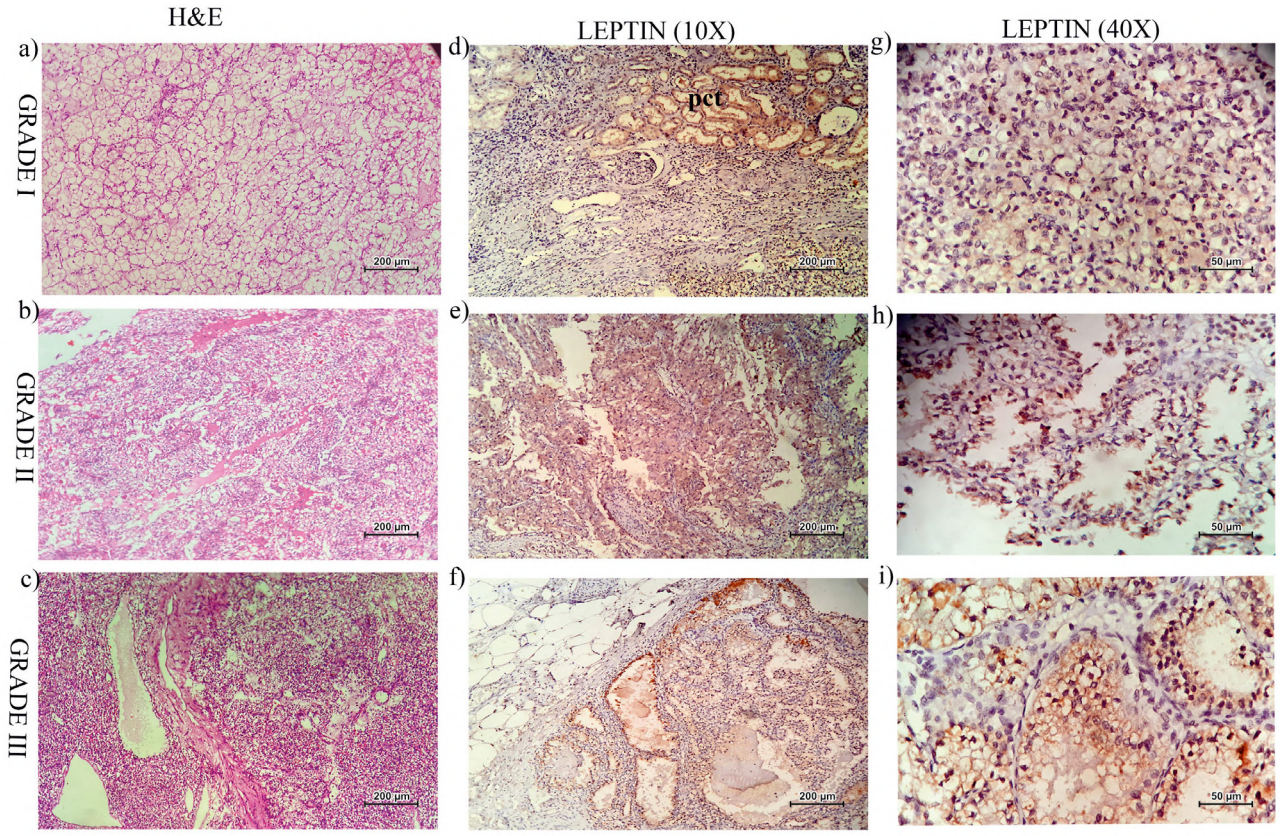

Fig. 4.- a), **b)** and **c)** show H&E sections of various grades of conventional renal cell carcinoma. **d), e), f), g), h)** and **i)** show increased expression of leptin in RCC tissues. Scale bars: a-f = 200 µm; g-i = 50 µm.

DISCUSSION

Studies on experimental animal models have revealed the potential benefit of reversing post-natal consequences by manipulating the leptin signalling pathways during early life stages. A study on maternal LP (low protein) rat models showed the effect of leptin administration enhanced the growth of the foetal pancreas. Similar results showed the growth of tissues such as kidneys, spleen, liver, and ovaries in the leptin-treated piglets with intra-uterine growth reduction. Though its expression has been demonstrated in the foetal tissues and foetal membranes its role still remains unclear (Reynolds and Vickers, 2019).

Leptin is thought to be produced by the placenta and secreted into the foetal and maternal circulation. Foetal leptin is also secreted by foetal adipose tissue and the foetal vascular endothelium (Tsai et al., 2015). The effect of leptin is largely through its transmembrane receptors having six spliced isoforms (OB-Ra-f). The positive expression of leptin in the PCT of human adult kidneys, as in the case of the current study, indicated its possible biological role in adult renal tissue. This could be attributed to the presence of the long form of the leptin receptor (OB-Rb) and short isoform OB-Raw with adequate signaling properties within the renal tissues, as stated by earlier studies. The OB-Rb is essential for intracellular signal transduction however the precise effect of the OB-Rb still remains to be explored (Ja Young Seol et al., 2007; Hoggard et al., 1997).

Contrarily, previous studies also show that with the exception of murine foetal brain and lung tissues, OB-Rb expression was not demonstrated in other tissues such as the liver, kidney, and adrenal gland even in their primitive forms (Hoggard et al., 1997). Thus, reflecting various roles of leptin in adult tissues that are redundant in the foetus.

A study conducted on leptin and various receptors in different segments of the nephron revealed the expression of leptin primarily in the PCT. The other components included the DCT, the loop of Henle, and the collecting duct (Hamma et al., 2004). A former study on rat models showed the PCT cells' uptake, internalization, and deg-

radation of 125I-leptin. The leptin is thought to be filtered by the glomeruli before metabolic degradation in the tubules. The study indicated the presence of megalin, a member of the low-density lipoprotein family at the apical membranes of PCT cells. The Megalin receptors bind to leptin in a Ca2+-dependent manner and tend to reabsorb, metabolize its ligand into endocytic compartments before recycling to the cell surface and thus help in leptin clearance. However, other tubular compartments or glomerular cells did not show the uptake of leptin (Hamma et al., 2004).

Leptin is known to be associated with tumorgenicity in several carcinomas. Leptin is bound to its Ob-RB receptor and is known to undergo a conformational change. This allows the activation of the Janus tyrosine kinase 2 (JAK2) pathway resulting in the phosphorylation of tyrosine residues of the receptor. It also activates the transcription 3 (STAT3) pathway (Ja Young Seol et al., 2007). While the long-form receptor is thought to signal through the Janus kinase pathway, the short-forms regulate the mitogen-activated protein kinase pathway (Tsai et al., 2015). It is also hypothesized to behave like a mitogenic agent in the cellular proliferation and differentiation of various types of cancer (Ja Young Seol et al., 2007). On the contrary, though the short isoform Ob-Ra is observed to activate mitogen-activated protein kinase (MAPK) signal transduction it fails to show proliferative behaviour or signal transduction in the peripheral tissues like the kidney (Harwick et al., 2001).

Studies show that serum leptin is an indicator of progression and survival in breast carcinoma. IHC expression of leptin in breast tumours of invasive ductal carcinoma is associated with tumour characteristics. It is used as a primary therapeutic target against breast carcinoma. Positive immunostaining of colorectal carcinoma (CRC) is associated with tumour size, lymph vascular invasion, distant metastasis, and recurrence in CRC (Al-Maghrabi et al., 2018). The decrease in serum leptin after colectomy indicates a definite association with CRC (Wang et al., 2016). Leptin is also associated with other Gastrointestinal (GI) tumours such as Barret's oesophagus and oesophageal adenocarcinoma (Clemons et al., 2013). Studies have observed that leptin is associated with an increased risk of progression of hepatocellular carcinoma, prostate carcinoma, pancreatic carcinoma, and gall bladder carcinoma (Jimenez-Cortegana et al., 2021).

Renal cell carcinoma (RCC) is the most common and accounts for 80-85% of malignant kidney tumours. Renal cell carcinoma is derived from the tubular lining epithelial cells of the kidney. The most common subtype of RCC is clear cell RCC (ccRCC) arising from the epithelial cells of PCT (Fan et al., 2021). Other subtypes of RCC are papillary cell RCC accounting for 15 to 20%, and chromophobe RCC accounting for 15% (Fan et al., 2021). A few proven etiological factors for RCC are alcoholism, tobacco smoking, obesity, hypertension, and genetic disorders like Von-Hippel Landau syndrome (Perumal et al., 2019). World Health Organization (WHO) has predicted that adiposity accounts for 24% of kidney malignancies (Ljungberg et al., 2011).

Studies performed with respect to serum leptin levels and RCC elucidated overlapping and controversial results. A case-control study done by Spyridopoulos et al. (2009) showed that low circulating leptin will lead to an increased risk of RCC while Liao et al showed that the more the serum concentration of leptin, more is the risk of RCC. The study could not explain the pro-carcinogenic effect of leptin in RCC (Spyridopoulos et al., 2009; Liao et al., 2013). Several other studies indicated serum leptin does not correlate with the progression of the disease in RCC (Perumal et al., 2019).

A few studies opined Acyl-coenzyme A: cholesterol acyl transferase (ACAT) is an important mediator that converts the free cholesterol into cholesterol esters inside the cell. Thus, the untoward (toxic) effects of free cholesterol on the tumour cells are controlled and thus have a protective effect on the latter. It is demonstrated that ACAT is increased in ccRCC when compared to normal renal tubular cells (Gebhard et al., 1987; Pinthus et al., 2011). Hongo et al. (2009) showed that ACAT activity is increased in the presence of leptin and proposed that leptin promotes RCC development. Studies also showed that there is no difference in the increase of leptin between clear-cell and non-clear-cell RCC and between the early and advanced stages of RCC (Drabkin

and Gemmill, 2010). According to a meta-analysis study, serum leptin was not associated with RCC. However, it was observed that the serum leptin levels are significantly elevated in non-clear cell RCC (Perumal et al., 2019). These authors showed that there is no relation between serum leptin and RCC development and progression (Perumal et al., 2019). However, in another study, no association was found between tumour characteristics and the IHC expression of leptin and its receptor. But the study found that nuclear expression of leptin was associated with overall survival (Zhu et al., 2018). Fan et al. (2021) studied RCC cell lines and Western blot and it was in accordance with similar results in the nuclear expression of leptin was associated with overall survival. The study by Ng et al. (2018) suggested that the level of Ob and ObR expression is higher in RCC than in normal tissues. It was observed that ccRCC shows strong cytoplasmic immunostaining. The above studies support our finding that PCT is specific for ccRCC. A study done by Perumal et al. (2020) demonstrated that there is no differential expression between ccRCC and adjacent kidney tissue. This was in contrary to our findings that demonstrated higher expression of leptin in ccRCC than in the adjacent tissue. Our study observed a graded increase in the expression of IHC from grade 1 to grade 3. While other studies done on breast cancer cell lines also showed high leptin association in high-grade/ stage tumors (Ishikawa et al., 2004). Further, the expression of leptin in other variants of renal neoplasms such as chromophobe, collecting duct carcinoma, and renal oncocytoma has been sparsely studied. Elesawy et al. (2020) observed that renal oncocytoma shows differential nuclear staining; chromophobe RCC shows no nuclear localization. Ng et al. (2018) demonstrated that nuclear expression of leptin is higher in renal oncocytoma compared to an eosinophilic variant of chromophobe RCC and an eosinophilic variant of clear cell RCC. Normally leptin stains mainly cytoplasm in non-cancerous tissues (Ng et al., 2018). The current study demonstrates that leptin is specific for PCT, we believe that the leptin biomarker can be used to distinguish the eosinophilic variant of chromophobe RCC, renal oncocytoma with Eosinophilic variant of clear cell RCC. Since the morphological overlap in renal malignancy is common, the scope for core needle biopsy (CNB) is limited. Leptin IHC can be used to distinguish the clear cell RCC from others. We emphasize that leptin IHC can be used pre-operative use in CNB will be valuable. In dilemma, it can also be used for the diagnosis of oncocytoma, chromophobe RCC, an eosinophilic variant of RCC.

CONCLUSION

The present study demonstrated leptin immunolocalized in the cytoplasm of the proximal convoluted tubules (+1) of the adult kidney and in the tumour cells (membranous) of conventional renal cell carcinoma (+2). However, leptin was not found to be localized in any of the renal components of the foetal kidneys. It was neither expressed in the nephrogenic zone nor neither in the developing proximal convoluted tubules. The proximal convoluted tubules are thought to be the source of the development of clear RCC. Thus, leptin may not participate in the development of human kidneys. Leptin expression in the PCT of the normal adult kidney, its enhanced expression in the PCT of the renal tissue adjacent to the carcinomatous tissue, and its overexpression in the cancerous cells indicate dysregulation of the leptin signalling pathways. Since the expression of leptin is observed to be absent in the fetal their expression in the renal tumor cells is indicative of an aberrant expression pattern unlikely to be due to reactivation of a repressed gene in the process of normal embryonic development.

ACKNOWLEDGEMENTS

The authors sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially increase mankind's overall knowledge that can improve patient care. Therefore, these donors and their families deserve our highest gratitude.

REFERENCES

AL-MAGHRABI JA, QURESHI IA, KHABAZ MN (2018) Expression of leptin in colorectal adenocarcinoma showed significant different survival patterns associated with tumor size, lymphovascular invasion, distant metastasis, local recurrence, and relapse of disease in the western province of Saudi Arabia. *Medicine*, 97: e12052.

BERGEN HT, CHERLET TC, MANUEL P, SCOTT JE (2002) Identification of leptin receptors in lung and isolated fetal type II cells. *Am J Respir Cell Mol Biol*, 27(1): 71-77.

BIESIADA LA, GŁOWACKA E, KREKORA M, SOBANTKA S, KROKOCKA A, KRASOMSKI G (2016) The impact of excessive maternal weight on the nutritional status of the fetus - the role of leptin. *Arch Med Sci*, 12(2): 394-401.

CLEMONS NJ, PHILLIPS WA, LORD RV (2013) Signaling pathways in the molecular pathogenesis of adenocarcinomas of the esophagus and gastroesophageal junction. *Cancer Biol Ther*, 14: 782-795.

DRABKIN HA, GEMMILL RM (2010) Obesity, cholesterol, and clear-cell renal cell carcinoma (RCC). *Adv Cancer Res*, 107: 39-56.

ELESAWY YF, AMER SI, EESA AN (2020) The reliability of leptin immunostain use for differentiating renal oncocytoma and chromophobe renal cell carcinoma. *Kasr Al Ainy Med J*, 26(1): 5.

FAN WL, YEH YM, LIU TT, LIN WM, YANG TY, LEE CW, LIN TC (2021) Leptin is associated with poor clinical outcomes and promotes clear cell renal cell carcinoma progression. *Biomolecules*, 11(3): 431.

FUHRMAN SA, LASKY LC, LIMAS C (1982) Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol*, 6: 655-663.

GEBHARD RL, CLAYMAN RV, PRIGGE WF, FIGENSHAU R, STALEY NA, REESEY C, BEAR A (1987) Abnormal cholesterol metabolism in renal clear cell carcinoma. *J Lipid Res*, 28(10): 1177-1184.

HAMA H, SAITO A, TAKEDA T, TANUMA A, XIE Y, SATO K, KAZAMA JJ, GEJYO F (2004) Evidence indicating that renal tubular metabolism of leptin is mediated by megalin but not by the leptin receptors. *Endocrinology*, 145(8): 3935-3940.

HARDWICK JC, VAN DEN BRINK GR, OFFERHAUS GJ, VAN DEVENTER SJ, PEPPELENBOSCH MP (2001) Leptin is a growth factor for colonic epithelial cells. *Gastroenterology*, 121(1): 79-90.

HOGGARD N, HUNTER L, DUNCAN JS, WILLIAMS LM, TRAYHURN P, MERCER JG (1997) Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. *Proc Natl Acad Sci USA*, 94(20): 11073-11078.

HONGO S, WATANABE T, ARITA S, KANOME T, KAGEYAMA H, SHIODA S, MIYAZAKI A (2009) Leptin modulates ACAT1 expression and cholesterol efflux from human macrophages. *Am J Physiol*, 297: E474-482.

ISHIKAWA M, KITAYAMA J, NAGAWA H (2004) Enhanced expression of leptin and leptin receptor (OB-R) in human breast cancer. *Clin Cancer Res,* 10(13): 4325-4331.

JA YOUNG SEOL, SOON YOUNG CHOI, NARI LEE (2007) Establishment of a stable cell line expressing mouse leptin receptor (OB-Rb) in human embryonic kidney 293 cell and preadipocyte 3t3l-1 cell. *Cancer Prev Res*, 12: 240-245.

JIMÉNEZ-CORTEGANA C, LÓPEZ-SAAVEDRA A, SÁNCHEZ-JIMÉNEZ F, PÉREZ-PÉREZ A, CASTIÑEIRAS J, VIRIZUELA-ECHABURU JA, DE LA CRUZ-MERINO L, SÁNCHEZ-MARGALET V (2021) Leptin, both bad and good actor in cancer. *Biomolecules*, 11(6): 913.

KAEMMERER D, PETER L, LUPP A, SCHULZ S, SÄNGER J, BAUM RP, PRASAD V, HOMMANN M (2012) Comparing of IRS and Her2 as immunohistochemical scoring schemes in gastro-enteropancreatic neuroendocrine tumors. *Int J Clin Exp Pathol*, 5(3): 187-194.

LAPPAS M, YEE K, PERMEZEL M, RICE GE (2005) Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies. *J Endocrinol*, 186(3): 457-465.

LIAO LM, SCHWARTZ K, POLLAK M, GRAUBARD BI, LI Z, RUTERBUSCH J, ROTHMAN N, DAVIS F, WACHOLDER S, COLT J, CHOW WH, PURDUE MP (2013) Serum leptin and adiponectin levels and risk of renal cell carcinoma. *Obesity (Silver Spring)*, 21(7): 1478-1485.

LJUNGBERG B, CAMPBELL SC, CHO HY, JACQMIN D, LEE JE, WEIKERT S, KIEMENEY LA (2011) The epidemiology of renal cell carcinoma. *Eur Urol,* 60(4): 615-621.

NG KL, DEL VECCHIO SJ, SAMARATUNGA H, MORAIS C, RAJANDRAM R, VESEY DA, WOOD ST, GOBE GC (2018) Leptin and its receptor: can they help to differentiate chromophobe renal cell carcinoma from renal oncocytoma? *Pathology*, 50(5): 504-510.

PATEL SR, DRESSLER GR (2013) The genetics and epigenetics of kidney development. *Semin Nephrol*, 33(4): 314-326.

PERUMAL K, HUIN WK, YAP NY, ONG TA, GOBE GC, RAJANDRAM R (2019) Role of leptin as a biomarker for early detection of renal cell carcinoma? No evidence from a systematic review and meta-analysis. *Med Hypotheses,* 129: 109239. Erratum in: *Med Hypotheses*, 131: 109284 (2019).

PERUMAL K, MUN KS, YAP NY, RAZACK AHA, GOBE GC, ONG TA, KUPPUSAMY S, RAJANDRAM R (2020) A Study on the immunohistochemical expressions of leptin and leptin receptor in clear cell renal cell carcinoma. *Biomed Res Int*, 4: 3682086.

PINTHUS JH, WHELAN KF, GALLINO D, LU JP, ROTHSCHILD N (2011) Metabolic features of clear-cell renal cell carcinoma: mechanisms and clinical implications. *Can Urol Assoc J*, 5(4): 274-82.

PRICE KL, LONG DA, JINA N, LIAPIS H, HUBANK M, WOOLF AS, WINYARD PJ (2007) Microarray interrogation of human metanephric mesenchymal cells highlights potentially important molecules in vivo. *Physiol Genomics*, 28(2): 193-202.

REYNOLDS C, VICKERS M (2019) The role of adipokines in developmental programming: evidence from animal models. *J Endocrinol*, 242: T81-T94.

SADASHIV R, BANNUR BM, SHETTY P, DINESH US, RANI H, VISHWANATHA J, DESHPANDE SK, BARGALE A, SARATHKUMAR, RUIKAR K (2019) Differential expression pattern of annexin A2 during nephrogenesis and kidney carcinoma. *Rom J Morphol Embryol*, 60(3): 895-904.

SPYRIDOPOULOS TN, PETRIDOU ET, DESSYPRIS N, TERZIDIS A, SKALKIDOU A, DELIVELIOTIS C, CHROUSOS GP (2009) Obesity and Cancer Oncology Group. Inverse association of leptin levels with renal cell carcinoma: results from a case-control study. *Hormones*, 8: 39-46.

TSAI PJ, DAVIS J, BRYANT-GREENWOOD G (2015) Systemic and placental leptin and its receptors in pregnancies associated with obesity. *Reprod Sci,* 22(2): 189-197.

WANG D, GAO L, GONG K, CHAI Q, WANG G (2016) Increased serum leptin level in overweight patients with colon carcinoma: A cross-sectional and prospective study. *Mol Clin Oncol*, 6: 75-78.

ZHU H, LI W, MAO S, WANG L (2018) Association between leptin level and renal cell carcinoma susceptibility and progression: A meta-analysis. *J Cancer Res Ther*, 14(4): 873-880.