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RESEARCH ARTICLE

High-Fat Diet-Induced Systemic Histopathological Changes in Adult Zebrafish: Implications for Oral Health and Nutri-tion.

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Abstract: ***Background***: Zebrafish is a superior vertebrate model to investigate particular genes and signaling pathways that or-chestrate kidney regeneration. For pre-screening purposes pharmaceutical companies use zebrafish. The kidney of zebrafish is similar to a mammalian kidney, it has nephrons, a glomerulus, proximal tubules, distal tubules, and collecting duct. Similarities of zebrafish kidney to that of mammalian organs is re-vealed by histological staining. Stains like H&E and PAS staining are used. The adult zebrafish kidney consists of arborized arrangements of nephrons that share common distal tube segments.***AIM -*** The aim of the study is to analyze the histopathological and histochemical analysis of adult zebrafish kidneys.***Materials and methods:*** 6 zebrafish samples were taken in which 4 were subjected to high fat and 2 were under control. These zebrafish were sacrificed and the kidney samples were fixed in 10 percent formalin. Routine H&E and IHC staining done.***Result:*** In subjected high fat zebrafish changes like increased lipid deposition, increased inflammatory cells, dilatation in glomerular capillaries, degeneration in nephrons, tubular defects were seen.***Conclusion:*** There was an enlargement of the kidney in the induced group when compared to the control groupEssential hypertension is a leading risk factor for cerebrovascular, renal and cardiovascular morbidity, left ventricular hypertrophy (LVH) is a key marker of adverse outcomes. Emerging evidence suggests vitamin D deficiency contributes to cardiac remodeling via modulation of the renin–angiotensin–aldosterone system, calcium balance, and endothelial function. ***Objective***: To evaluate the correlation between serum vitamin D levels with left ventricular mass index (LVMI) and its parameters in patients with essential hypertension. ***Methods***: A hospital-based observational study was conducted on 100 patients with essential hypertension at MMIMSR, Ambala (January 2023–March 2025). Demographic, clinical, and biochemical data were collected. Serum 25(OH)D was measured and classified as deficient (<20 ng/mL), insufficient (20–30 ng/mL), or sufficient (≥30 ng/mL). Echocardiography was performed to assess LVMI, interventricular septal thickness (IVST), posterior wall thickness (PWD), and end-diastolic diameter (EDD). Statistical analyses included ANOVA, Chi-square, and Pearson's correlation. ***Results***: Vitamin D deficiency was highly prevalent (62%), with an additional 20% insufficient. Abnormal LVMI was found in 66% overall, with higher prevalence in the deficient group (71%) compared to insufficient (60%) and sufficient (55.6%), though not statistically significant (p = 0.391). Abnormal PWD was significantly associated with vitamin D deficiency (96.8% vs. 77.8%, p = 0.031). IVST and EDD showed no significant associations. Proteinuria was more frequent in deficient patients (p = 0.038), reflecting renal target organ damage. ***Conclusion***: In India Vitamin D deficiency is widespread in general population, however it is significantly more among hypertensive patients and is associated with adverse echocardiographic changes, particularly posterior wall thickening. While LVMI and IVST trends indicated higher values in deficient groups, statistical significance was not reached. These findings highlight vitamin D as a potentially modifiable factor in reducing hypertensive target organ damage and cardiovascular risk.

Keywords: Histopathological, histochemical, zebrafish kidney .

INTRODUCTION

Zebrafish belong to the minnow family and are an excellent vertebrate model for studying specific genes and signaling pathways that orchestrate kidney regeneration. Zebrafish are easy to examine and are unique model animals for biomedical research [1]. The kidney of zebrafish is similar to a mammali-an kidney, it has nephrons, a glomerulus, proximal tubules, distal tubules and collecting duct. Similari-ties of zebrafish kidney to that of mammalian organs is revealed by histological staining [2]. It has a fully sequenced genome, high fecundity, genetically manipulatable, nearly transparent embryo, rapid development and external fertilization. Zebrafish is also being used in various fields of research such as cardiology for the study of heart regeneration mechanisms [3].

Since the first use of zebrafish as an experimental model in 1955, it has been consolidated as a model organism for biology, genetics, pharmacology, and general biomedical research. The exponential growth of zebrafish use in laboratories is due to several favorable features such as its fast life cycle (the adult stage reached about 6 months), high fecundity (hundreds of eggs are laid in each mating), trans-parency of the embryonic/larval stages which facilitates the observation of the development, low maintenance cost, and easy handling [4]. Due to its small size and low body weight, it requires a rela-tively low quantity of the compound tested, which is an excellent advantage in the research [5].Despite the phylogenetic distance, zebrafish have about 70% of genetic homology toward humans. Hence, it is considered a reliable tool for preclinical studies [6].

The zebrafish kidney lies in a retroperitoneal location, just ventral of the vertebral column. It has dis-tinct head and trunk regions. Similar to the mammalian kidney, it has nephrons with a glomerulus, proximal tubules, distal tubules, and collecting ducts. However, the distal tubules are difficult to dis-tinguish from the proximal tubules with routine hematoxylin and eosin (H&E) staining . The renal in-terstitium contains hematopoietic cells. Endocrine cells (interrenal and chromaffin cells) can be found along the major blood vessels in the anterior part of the kidney [7,8].

Histopathology is useful to detect potentially deleterious effects of compounds to a particular organ or tissue. The choice of the organ depends on the type of compound tested and the metabolism of the an-imal used. Zebrafish is an excellent model to perform histopathology assessment [9]. Several organs of zebrafish have been used in toxicological studies, such as the gonads, pharynx, thyroid, intestine, liver, kidney, gills, and muscles. A considerable advantage of zebrafish in histopathology is the possibility to observe several organs in only one slide due to its small size [10]. The kidney of zebrafish is accounta-ble to excrete the excess of water that enters through the mouth; it also has a role in the filtration of residues and osmotic balance [11]. This organ shares characteristics with those of animals and is one of those most impacted by hazardous substances [12]. It is clear that we have to understand the normal histopathological and histochemical pattern of the zebrafish kidney to compare it to the specimen that has been subjected to toxicity. Thus, the aim of the study is to analyze the histopathological and histo-chemical analysis of adult zebrafish kidneys.

MATERIALS AND METHODS

6 zebrafish samples were taken in which 4 were subjected to high fat and 2 were under control. These zebrafish were sacrificed and the kidney samples were fixed in 10 percent formalin.
Staining protocol:

The sections were deparaffinized using Xylene for 20 minutes and rehydration was done using alcohol for 10 minutes. Sections were washed in running tap water for 3-5 minutes and were stained with Har-ris's haematoxylin for 5 minutes, washed in running tap water; differentiation was achieved by
dipping the slides in acid alcohol for one dip, then dipped in ammonia for one dip and washed in run-ning tap water for bluing. Slides were transferred to eosin for a single dip after which the slides were dehydrated through descending grades of alcohol; the slides were cleared in xylene and mounted with DPX.

Data Collection
The slides were viewed under the microscope by two independent blinded observers and the depth of invasion was noted and tabulated. The depth of invasion was measured using the magna software, and the patient's information was recorded using the patient records. Measurement was done by experi-enced pathologists in millimeters

RESULTS

In this study 6 zebrafish samples were taken, 4 were subjected to a high fat diet and 2 were under con-trol. In the high fat diet group it was noticed that there is an increased lipid deposition when compared to the control group, dilatation in glomerular capillaries and other blood vessels was detected using SMA, degeneration in nephrons, segmental necrosis and tubular defects were also noticed. There was thickening in the basal membrane of glomeruli and increased inflammatory cells in the high fat diet group as compared to the control group. There was an enlargement of the kidney in the induced group when compared to the control group.

The group statistics of high fat diet and controlled group of lipid, degeneration, proliferation, inflam-mation is shown below in the table 1. The group statistics show that there is an increase in lipid, degen-eration, proliferation and inflammation in the high fat diet group when compared to the controlled group.

	GROUP	MEAN	STD. DEVIATION	STD. ERROR MEAN
Lipid	Control	1.0000	.00000	.00000
	Induced	2.7500	.50000	.25000
Degeneration	Control	1.5000	.70711	.50000
	Induced	1.7500	.50000	.25000
Proliferation	Control	1.5000	.70711	.50000
	Induced	1.7500	.50000	.25000
Inflammation	Control	1.5000	.70711	.50000
	Induced	2.0000	.00000	.00000

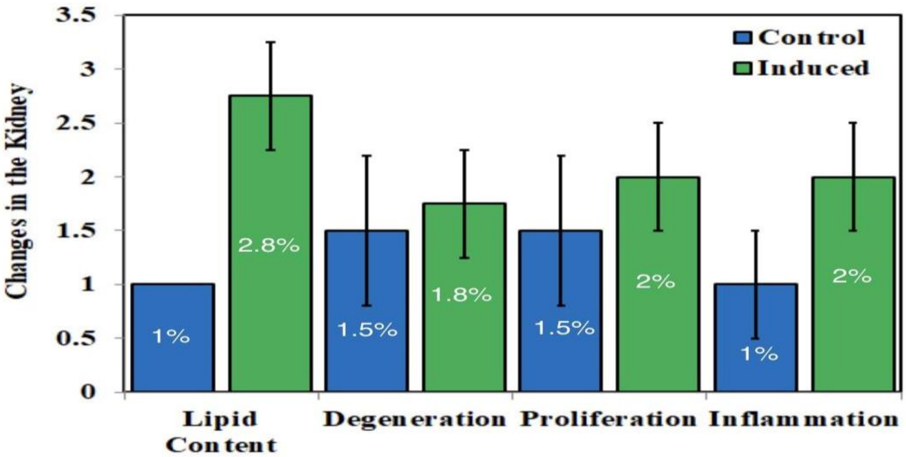

Figure 1: The bar chart illustrates the mean value of lipid content , degeneration, proliferation and inflammation percentage in both control and induced zebrafish. X axis represents the control and induced group. Y axis represents the mean percentage of changes in the kidney . The blue color denotes the controlled group percentage and the green color denotes the induced group percentage.

The mean value of the controlled group and induced group count is 1.00 ± 0.00 and 2.75 ± 0.50 respectively.

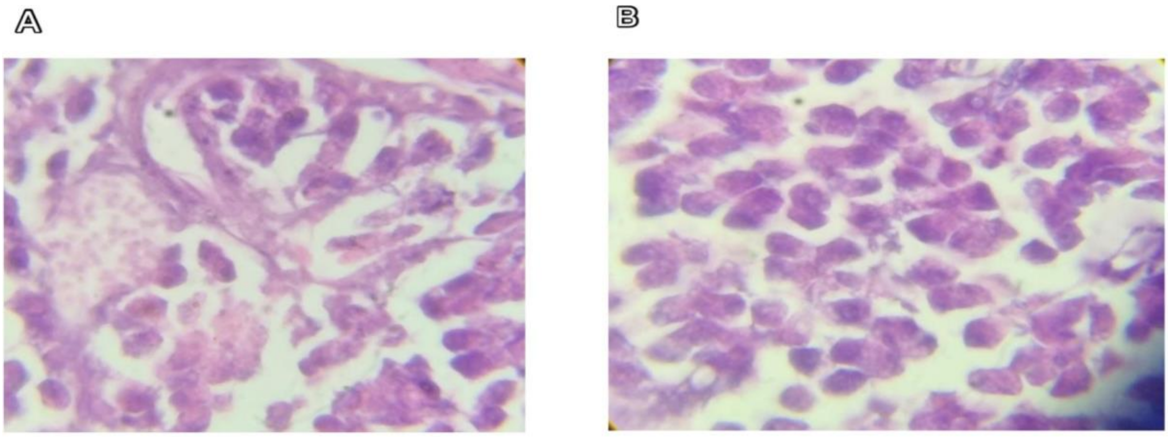

Figure 2: A) Control kidney tissue samples B) The test samples kidney tissue

DISCUSSION

Zebrafish kidney is similar to mammalian kidney as it has nephrons, a glomerulus, proximal tubules, distal tubules and collecting duct and is revealed by histological staining. In the present study it is found that there is an enlargement in the kidney of a high fat diet zebrafish. Similarly in a previous study it was reported that in a high fat diet zebrafish, changes are observed in glomeruli, tubular diam-eter has been increased, tubular epithelial cells and tubular lumen are enlarged [13]. In a previously conducted study it has been reported that approximately 70 percent of the human gene have at least one clear zebrafish orthologue when compared with the human reference genome [14].

Due to zebrafish genetic tractability, it provides an advantage to discover the signaling events and pathways involved in kidney regeneration [15]. The pronephric kidney of zebrafish provides a helpful and pertinent model of kidney development and function. It is made up of cell types that are present in all vertebrate kidneys, and transcription factors that are highly conserved in mammalian kidney devel-opment control pronephric organogenesis [16]. Zebrafish models are also used in several researches for studying human diseases such as Parkinson’s, cancer, heart disease, Alzhemer’s and muscular dystro-phy since it's an excellent vertebrate model [17]. In a previous study conducted it is reported that the zebrafish fed a high calorie diet and independent of fats macronutrient composition, the zebrafish exhibited glomerulomegaly, foot process effacement, and filtration barrier failure, mirror-ing the abnormalities found in obese people. It reports glomerular basement membrane thickening, tub-ular enlargement of $p < 0.0001$ [13]. This suggests that the macronutrient composition is less important than the total calories in obesity related kidney disease.

Similarly, in a study conducted previously it is reported that zebrafish subjected to a high fat choles-terol diet has become obese and show an increase in deposition of adipose tissues in abdominal area [18]. Obesity is associated with fat intake, in humans a high fat diet can induce obesity and its risk fac-tors. In a recent study conducted it is reported that the zebrafish under a high fat diet developed an in-crease in BMI, adipose tissue volume, glucose intolerance and plasma triglyceride [19,20]. In the pre-sent study it is shown that there is an increased lipid deposition when compared to the controlled group, dilatation in glomerular capillaries and other blood vessels was detected using SMA. Also, zebrafish subjected to a high fat diet showed degeneration of nephrons, segmental necrosis and tubular defects and an increase in inflammatory cells were observed. It was observed that there was an en-largement of the kidney in the induced group when compared to the controlled group. Due to the time constraint the sample size was limited, a larger sample size along with special stains and immunohisto-chemistry and immunofluorescence can be explored for better results.

CONCLUSION

To conclude, there is an increase in lipid deposition, degeneration in nephrons, segmental necrosis and tubular defects, dilatation in glomerular capillaries and other blood vessels were observed using SMA in a zebrafish subjected to high fat diet zebrafish. These are the risk factors that can occur in the mam-malian kidney who are subjected to a high fat diet since zebrafish kidney is similar to mammalian kid-ney.

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