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Statin Induced Changes in Rat Pancreatic Tissue: A Histological and Biochemical Perspective

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Abstract

Objective: This study aimed to elucidate the effects of simvastatin on pancreatic histomorphology and biochemical profile in a rat model.

Method: Forty male Sprague-Dawley rats weighing 250 g were divided into two equal groups: a control group (A) and an experimental group (B) treated with simvastatin 60 mg/kg/day for 12 weeks. At the end of the experiment, the rats were euthanized, and their pancreas was collected for analysis and examined using gold-standard histological parameters including inflammation and fatty infiltration. Biochemical parameters i.e. serum amylase and alkaline phosphatase were also analysed. Statistical Package for the Social Sciences (SPSS) software version 23 was employed for data analysis. Mean ± SD was employed to express quantitative variables. Qualitative variables were expressed as frequencies and percentages. Chi-square tests compared qualitative variables while independent sample t-tests compared quantitative variables between groups, with $P < 0.05$ defining statistical significance.

Results: The administration of Simvastatin resulted in a substantial induction of pancreatic inflammation and fatty infiltration, accompanied by notable alterations in the biochemical profile. Specifically, there was a significant reduction in serum amylase levels and a concomitant increase in serum alkaline phosphatase (ALP) levels, as compared to the control group.

Conclusion: This study demonstrates that simvastatin profoundly alters pancreatic histomorphology in rats, resulting in marked inflammation, necrosis, fatty infiltration, and interstitial fibrosis. These novel findings provide unique insights into simvastatin's effects on pancreatic morphology using a robust animal model.

Keywords: Simvastatin; Sprague-Dawley rats; pancreatic histomorphology, Amylase, Alkaline phosphatase.

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1. Introduction

Statins revolutionized dyslipidemia management, but concerns persist over their side effects. Myopathies are common, but statins' effects on the pancreas histomorphology and biochemical profile remain poorly defined.[1] The pancreas regulates glucose homeostasis via insulin and glucagon secretion by endocrine islets, while acinar cells supply digestive enzymes. Disrupting this delicate balance can impair exocrine and endocrine function.[2]

Studies have linked statin use with pancreatitis, purportedly from inflammatory responses, cellular toxicity, or toxic metabolite accumulation. However, evidence remains mixed, with some studies showing no association with statins.[3] Histomorphological changes underlying this possible risk are poorly understood. This research yields a significant and vital impact on understanding and observing statin-induced histological changes in the structure and exocrine function of the pancreas of rats. We demonstrated the comprehensive histological and biochemical analysis delineating statins' pancreatic impact. Statin-induced pancreatitis has been seen in case reports, control studies, and meta-analyses. Statin-treated rats exhibited laboratory-based biochemical evidence and histomorphological changes representing the visual evidence of direct pancreatic toxicity. These structural anomalies disrupted pancreatic physiology, providing a morphological basis for pancreatitis development. Fatty insinuation in the pancreas may also lead to diabetes mellitus, and pancreatitis and may be a predisposing cause of pancreatic cancer.[4]

The results of this study will be of paramount importance for therapeutic strategies in patients on long-term treatment of statins.

2. Materials & Methods

This innovative research study was conducted in the Department of Anatomy, Army Medical College Rawalpindi, teaming up with two prestigious research institutes i.e. National Institute of Health (NIH)

Islamabad and Armed Force Institute of Pathology (AFIP) Rawalpindi. Utilizing a laboratory-based investigational control trial spanning one year, the study design was reviewed and approved by the Ethical Committee on Animal Experiments of Army Medical College, Rawalpindi before commencement.

Forty male Sprague-Dawley rats weighing 250 g were divided into two equal groups: a control group (A) and an experimental group (B) treated with simvastatin for 12 weeks. The control group (A) received a standard diet and water for three months through an oral gavage tube, while the simvastatin group (B) received a standard diet supplemented with 60 mg/kg/day[5] of simvastatin daily orally for three months through a gavage tube; at the end of the experiment, the rats were euthanized, and their pancreases were collected for analysis. A 5 ml sample of blood was collected through cardiac puncture[6] in a plain test tube for the quantitative measurement of amylase and alkaline phosphatase levels from the animals in both groups.

The inflammation was recorded as present or absent in X40 objective magnification. It was recorded as present based on the presence of polymorphonuclear cells and neutrophils in the pancreatic acinus and islets. Global fatty infiltration scores were calculated through the cumulative scoring of per lobular and intralobular fat severity.[7]

Statistical Package for the Social Sciences (SPSS) software version 23 was employed for data analysis. Qualitative variables were expressed as frequencies and percentages. Chi-square tests compared qualitative variables between groups, with $p < 0.05$ defining statistical significance. Mean ± SD was employed to express quantitative variables. Shapiro Wilk test was used to check data distribution (data was found to be uniform or nonskewed). Independent sample t-test was applied to determine the statistical significance between the groups.

3. Results

The control group (A) did not display marked structural pancreatic changes (Fig 1). Inflammation appeared only in 1 rat (5%). Perilobular and intralobular fatty infiltration occurred in 5 (25%) and 2 (10%) rats, respectively, although no global fatty infiltration was observed.

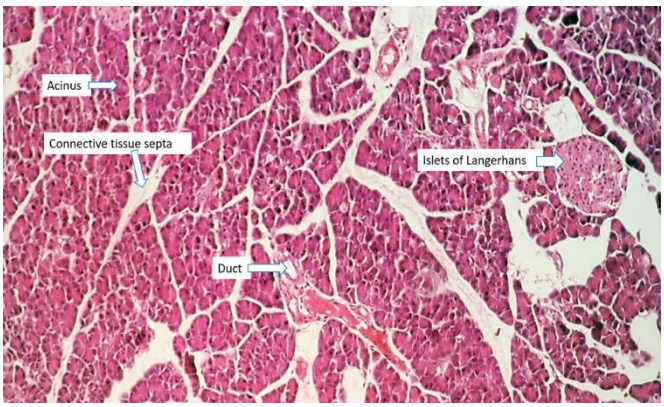

Figure 1: Photomicrograph of histological section of pancreas of rat in control group showing normal histology. (Acinus , duct , islets of Langerhans and connective tissue septa)

Conversely, the experimental group (B) exhibited marked pancreatic alterations (Figure 2). Inflammation with immune cell infiltration affected 15 rats (75%), representing a significant increase versus controls ($p<0.002$). Perilobular and intralobular fatty infiltration were present in 13 (65%) and 9 (45%) rats, respectively (Fig 2).

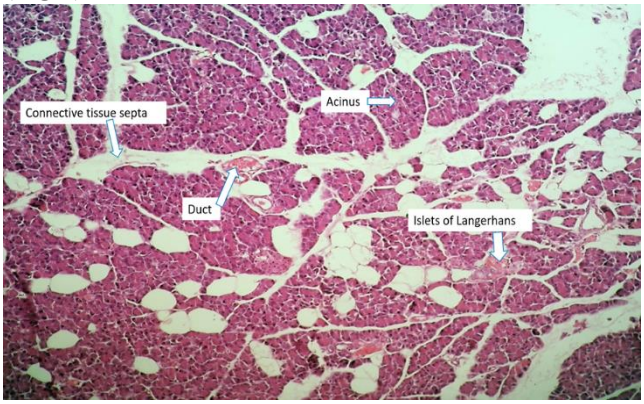

Figure2: Photomicrograph of histological section of pancreas of rat in B group showing fatty infiltration. (Acinus , duct , islets of Langerhans and connective tissue septa)

Global fatty infiltration was confirmed in 6 rats (30%), also significantly higher than controls ($p=0.020$).

Quantitatively the serum amylase levels were significantly reduced in group B (953±94 U/L) as compared to group A (1557±316 U/L) with p-value=0.004. Serum ALP levels surged in group B as compared to control as shown in table 1.

Table 1: Comparison of inflammation, fatty infiltration and biochemical markers between the control and experimental group

Parameter	Control group A	Experimental group B	p-value
Inflammation present	1 (5%)	15 (75%)	0.002*
Fatty infiltration present	0 (0%)	6 (30%)	0.020*
Serum Amylase (U/L)	953±97	1557±316	0.004*
Serum ALP (U/L)	66±21	101±39	0.003*

In summary, simvastatin induced a constellation of pancreatic histological changes in rats, particularly inflammation and fatty infiltration and concomitant changes in biochemical markers. This data provides unique insight into the pancreatic effects of simvastatin in vivo model.

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4. Discussion

The histomorphological analysis reveals striking pancreatic alterations in simvastatin-treated rats. Perhaps most strikingly, inflammation increased dramatically with simvastatin, affecting 75% of treated rats versus just 5% of controls. Immune cell infiltration signifies direct drug-induced injury. Fatty infiltration was also significantly more prevalent, with 65% and 45% of experimental rats exhibiting per lobular and intralobular adiposity respectively. Global replacement by fat occurred in 30%, consistent with lipomatosis development,[8] Together, these findings provide unique visual evidence that simvastatin disrupts pancreatic morphology. The inflammation and fatty changes are particularly noteworthy, as they can disturb exocrine and endocrine function.[9] The presence of immune cells and adipocytes in the parenchyma alters the pancreatic microenvironment. This may impair acinar enzyme production and islet hormone secretion, providing a histopathological basis for clinical reports of statin-induced pancreatitis and diabetes.[10] Notably, the patterns observed here evoke the initial changes in type 1 diabetes pathogenesis. Lymphocytic infiltration and lipomatosis mark the early stages, followed by progressive islet destruction.[11] Though our 8-week study did not demonstrate advanced islet loss, the precocious development of inflammation and fatty replacement raises concerns about prolonged statin use. Surveillance of pancreatic structure and function in long-term statin users is warranted.

Obesity leads to an increase in the weight of the pancreas by inducing lipomatosis in the pancreas. Lipomatosis of the pancreas is a condition which is characterized by the accumulation of adipose cells in and around it.[12] It has been found that intralobular and per lobular fat accumulation in rat pancreas is associated with impaired hormone/enzyme secretion. The current study is comparable with the study based on human data that obesity leads to an increase in pancreatic volume as well as pancreatic fat accumulation.[8]

The results of the current study are comparable with the steps involved in the process of development of Insulin-dependent diabetes mellitus (IDDM) in rats. The pancreas histopathological findings in IDDM are divided into three stages: an early stage which shows mild lymphocytic infiltration in and around serous acinus part and islets of Langerhans. In the middle stage, there is damage to islet cells with fibrosis and necrosis of islets along with the formation of new islets of Langerhans to compensate for insulin release.[13] Another histological finding in this stage is hyperplasia of islets of Langerhans. During the final stage there is the destruction of islets of Langerhans with replacement of endocrine cells with fibrotic tissue,[14] In severe cases there is atrophy of pancreatic tissue while fatty and fibrotic tissue completely replaces the healthy pancreatic tissue. The islets are completely lost in this final stage,[15] Histopathologically, the presence of inflammation, fatty changes, increased number of islets of Langerhans and hypertrophy of islets are directed towards the initial stages of development of IDDM.[16] However, the time duration of the current study was not long enough to show the final stages of the development of IDDM.

Lower serum amylase values in the experimental group with fatty pancreas possibly reflected diffuse destruction of the pancreas due to fat infiltration.[17] This ectopic deposition of fat in the pancreas has been linked to the

development of insulin resistance, type II diabetes mellitus, and pancreatic cancer.[18] Despite its clinical relevance, our understanding of pancreatic fat content and its implications on the clinical profile remains limited.

Higher ALP levels may be explained as a result of initial stages of chronic pancreatitis which lead to partial common bile duct obstruction, increasing biliary pressure and causing dilation of the proximal bile duct.[19] This partial obstruction induces the synthesis of hepatic alkaline phosphatase, which subsequently regurgitates into the serum due to impaired bile flow.[20] This phenomenon highlights the intricate interplay between pancreatic and biliary physiology, providing insights into the mechanisms contributing to pancreatitis.[21] Further research is needed to explore the complex molecular pathways involved in the causation of this phenomenon.

Recommendations:

We recommend that further studies are needed to elucidate how statins exert direct pancreatic toxicity. Clinical studies should examine correlations between statin dose/duration and more robust biochemical markers of exocrine and endocrine dysfunction. Prescribers should closely monitor patients on statins for symptoms of pancreatitis and screen for new-onset diabetes, especially with long-term use.

Limitations: This short duration cannot assess long-term pancreatic effects from chronic statin use. This rat model may not fully replicate effects in human patients. Clinical-pathological correlation studies are needed to confirm translational relevance.

It is obvious from the discussion that; these structural distortions provide unique visual evidence that statins disrupt pancreatic integrity. The inflammation and fatty replacement are particularly striking, as they can disturb the organ's delicate exocrine and endocrine functions which is evident from biochemical markers assays.

5. Conclusion

This study demonstrates that statins profoundly impact pancreatic histomorphology and biochemical parameters. Simvastatin-treated rats exhibited a concerning constellation of changes, including significantly increased inflammation, congestion, and fatty infiltration compared to controls. Worrisome trends also emerged in chemistry analysis.

CONFLICTS OF INTEREST- None

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Contributions:

A.Q - Conception of study
A.Q - Experimentation/Study Conduction
F.M - Analysis/Interpretation/Discussion
A.Q, H.G.K - Manuscript Writing
M.M.K, M.R.B.K - Critical Review
H.K - Facilitation and Material analysis

All authors approved the final version to be published & agreed to be accountable for all aspects of the work.

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