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RESEARCH ARTICLE
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Effect of Folic Acid on Avascular Bone Necrosis in mice Associated with Hypercholesterolemia

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ABSTRACT

To comprehend the etiology of avascular necrosis better, many experimental models are available. Femoral head osteonecrosis, commonly known as avascular bone necrosis. is a condition with a complex origin that is defined by a significant alteration in the structure of the bones, which lowers bone resistance and causes femoral head collapse. Between February and April 2022, this study was conducted with the goals of creating a mouse model for the production of avascular necrosis (AVN) using HFD and examining the impact of folic acid on bone health. Lipid indices were found to assess how FA affected the mice's HFD-fed lipid metabolism. HE staining was used to detect morphological and structural changes in the bone. The findings of this study demonstrated that AVN had a malfunction of lipid metabolism. The mean levels of total cholesterol (TC), triglycerides (TG), and low density lipoprotein cholesterol (LDL-C) in the AVN group were significantly greater than those in the control group. Serum triglycerides were decreased in groups with FA supplementation, with the most pronounced reduction in the groups of mice that take folic acid before one week from taking HFD. The mean Low density lipoprotein cholesterol (LDL-C) values were significantly lower in the AVN + folic acid group compared with those in the HFD control group. The HFD control group's histological investigation using hematoxylin and eosin staining revealed necrotic bone, lacunae containing necrotic osteocytes, and multiple cavities that indicated lack of content, a sign that the osteocytes inside had died. Chondrocyte degradation was seen in addition to necrotic bone marrow. Additionally, it was discovered that the majority of bone trabeculae had necrotic lesions that had damaged them, and that the femur had a buildup of lipid droplets. Both folic acid-treated groups showed a decrease in osteonecrosis and an improvement in the lesion in the trabecular bone tissue.

Keywords: *Folic Acid, Avascular Bone Necrosis, Hypercholesterolemia*

INTRODUCTION

A vascular necrosis, commonly referred to as osteonecrosis (ON), is characterized by the cellular death of bone components brought on by a blood supply disruption that may cause joint deterioration and ischemia. Although the precise process causing ON is yet unknown, several possible culprits include mechanical stress, intraosseous microcirculation coagulation, ischaemia, and vascular occlusion [1]. Trauma, alcoholism, smoking, vascular disease, renal illness, coagulation disorders, rheumatic diseases, including systemic lupus erythematosus (SLE), as well as medical conditions like

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hypercholesterolemia are just a few of the risk factors and medical conditions connected to ON. [1]

The relationship between AVN and cholestrolaemia is still debatable, albeit [2]. According to earlier research, hyperlipidaemia-induced fat embolisms may have contributed to the development of AVN[3].

A water-soluble vitamin called folic acid (FA) is crucial for controlling blood lipids and antioxidants. [4]

The functions of folic acid include regulating lipid metabolism [4], correcting aberrant DNA methylation [4], and controlling the phenotype of monocytes. So, in the current study, we looked into how folic acid protects against AVN.

However, little is known about the role of FA and its underlying processes in osteonecrosis brought on by an HFD. [5]

Therefore, the purpose of the current investigation is to determine whether FA from HFD-fed mice could control lipid metabolism as a starting point for nutritional intervention strategies for HFD-induced vascular bone necrosis.

In this investigation, we examined the hypothesis that lowering serum triglyceride levels with folic acid in treated mice will result in decreased a vascular bone necrosis

MATERIALS AND METHODS

Animal experiments

Twenty female mice aged 10 weeks were purchased from Laboratory Animal of college of science , university of al kufa .

After 1 wk of adaptive feeding, mice were randomly divided into five groups:

1- First group the mice were fed folic acid for one week then a HFD+ folic acid (88% standard diet, 10% vegetable oil, and 2% cholesterol) for 8 wk.

2-The second group fed an HFD with folic acid (40 mg/kg·d) for 8 wk.

3- Third group with HFD only(88% standard diet, 10% vegetable oil, and 2% cholesterol) for 8 wk.

4- The forth group fed normal diet + folic acid (40 mg/kg·d) for 8 wk.

5- The fifth group with normal diet as control negative for 8 wk. .

Biochemistry

Blood samples were taken after 8 weeks of feeding, standing at 4°C for 6–8 hours, then being centrifuged at 3000 rpm for 15 min. The serum was then carefully transferred to a clean EP tube.

An automatic biochemistry analyzer was used to measure the levels of serum triglycerides (TG), total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL).

Histological Assessment

Mice were slaughtered, and the femurs were removed. The femurs were then preserved in 10% neutral buffered formalin for two to three days. Bones were soaked in EDTA for three weeks to decalcify them. Specimens were processed using a graded alcohol series before being embedded in paraffin, cut into 4 m-thick pieces, and stained with hematoxylin and eosin (H&E) for pathological examination [6]. To examine the clinical biochemical data from various groups, one-way ANOVA was used.

RESULTS

Effect of FA on Lipid Metabolism Induced by HFD in Mice

Mice were examined for the impact of FA on the blood lipids of HFD-fed mice in order to investigate the effect of FA on lipid metabolism abnormalities caused by HFD.

The findings demonstrated that the HFD group's serum levels of TG, TC, and LDL-C were significantly greater than those of the control group, whereas serum levels of TG, TC, and LDL-C were decreased following FA intervention (Table 1) The TC, CH, HDL, and LDL levels in the AVN group were considerably higher than those of the control group at 204 1.73 mmol/L, 194.5 1.73 mmol/L, 52.7 1.38 mmol/L, and 100 1.73 mg/dL, respectively, while the

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levels in the HFD and FA group (1) were 90 2.88 mmol/L and 66 2.88 mg/dL

TABLE 1: comparison of average blood lipid levels between groups

VLDL(mmol/L)	LDL(mmol/L)	HDL(mmol/L)	CH(mmol/L)	TG(mmol/L)	GROUPS
18	92	66	176	90	GROUP 1
26.8	41.9	58.3	127	134	GROUP 2
40.8	100.95	52.75	194.5	204	GROUP 3
38	1.75	86.75	126.5	190	GROUP 4
33.6	49.1	74.3	157	168	GROUP 5

Histopathology

The trabecular architecture of control bones was normal, and the marrow had normal cartilage cells without any necrosis (Fig. 7). In contrast, AVN bones had several necrotic areas, indicating significant damage (Fig3). the cartilage cells seemed damaged with vacuolar degeneration, and there were apparent hollow holes between the chondrocytes, indicating a loss of tissue structure (Fig4).

A number of empty lacunae caused by the loss of osteocytes are also seen in the AVN bone sections (Fig. 3).

Additionally, lipid droplets of various sizes were seen in the bone of the HFD animals, showing that the bone was infiltrated with fat when necrosis occurred. The number of adipocytes in the HFD + FA groups was lower than that in the HFD group (Fig2)

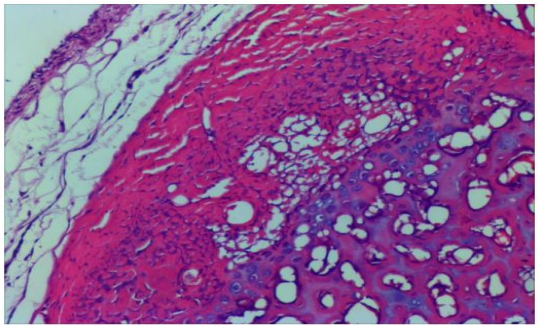

FIGURE 1: Trabecular bone tissue in group 3 with numerous empty bone cavities, because of the death of osteocytes inside (HE staining, ×100).

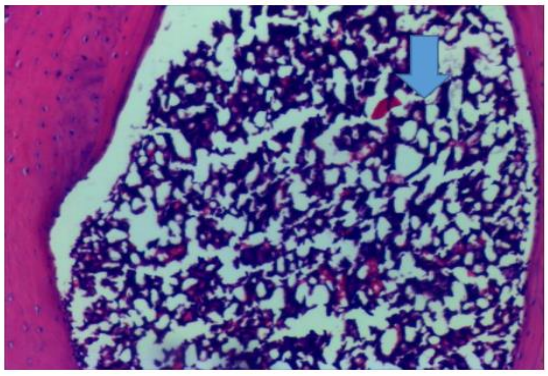

FIGURE 2: bone loss and destruction induced by high-fat diet in group 3, blue arrows point to fat accumulation in the femoral head (HE staining, ×100).

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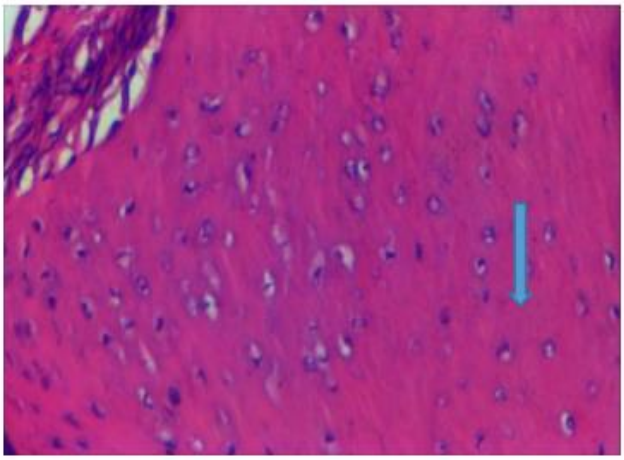

FIGURE 3: trabecular bone tissue in group 3 with numerous empty bone cavities, because of the death of osteocytes inside (blue arrow) (HE staining, ×200).

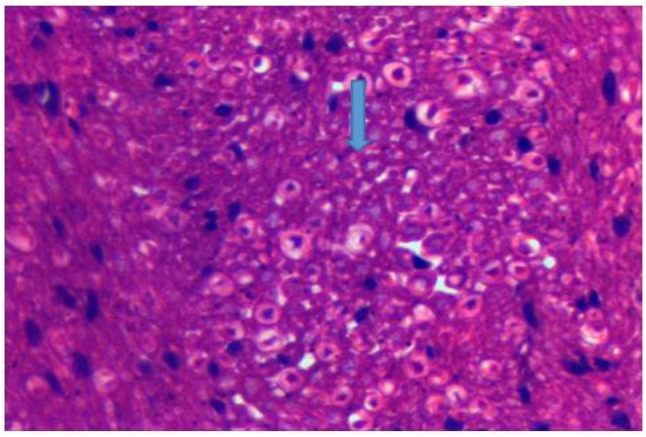

FIGURE 4: The Blue arrow points to the site where vacuolar degeneration occurred in group 3(HE staining, ×400).

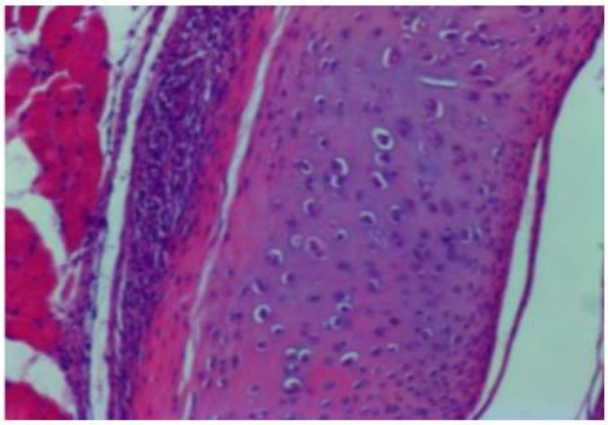

FIGURE 5: FA improves bone loss and destruction induced by high-fat diet in group 2 (HE staining, ×100).

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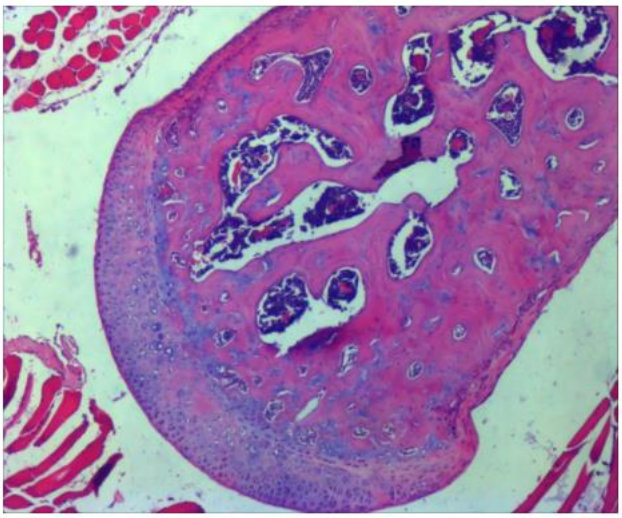

FIGURE 6: FA improves bone loss and destruction induced by high-fat diet in group 1 (HE staining, ×40).

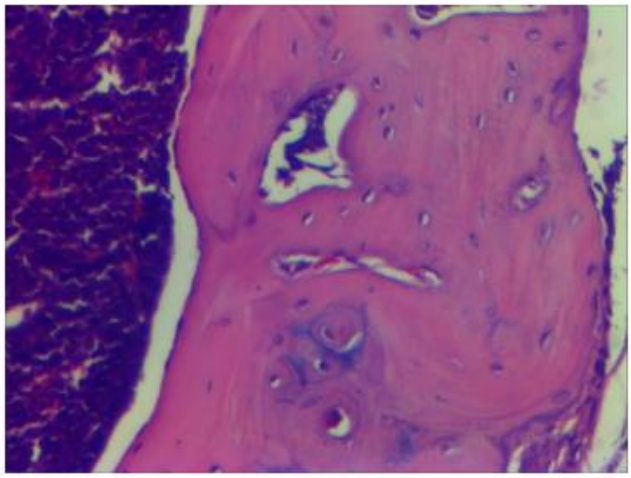

FIGURE 7: Control mice were fed with the CD with normal histological feature(HE staining, ×200).

DISCUSSION

Although the cause of osteonecrosis is still not fully understood, lipid metabolism has been shown to be involved [7].

One of the causes of avascular necrosis, which is primarily related to hypercholesterolemia, has been identified as a disturbance of lipid metabolism. [8]

There haven't been many research, though, looking into whether decreasing triglycerides with medication can stop osteonecrosis.

Finding ON treatments that can manage the joint microenvironment presents a special challenge.

In order to investigate the impact of FA on HFD-induced osteonecrosis, we created a mouse model of osteonecrosis caused by HFD.

Our findings demonstrate that FA decreased the metabolic conditions brought on by an HFD, including hyperlipidemia.

A long-term high-fat diet encourages adipogenesis and prevents bone growth. Adipocytes and osteoclasts are both produced from bone marrow mesenchymal stem cells. [8].

According to several reports, HFD increases bone resorption and reduces bone formation [9]. Due to an excessive development of adipose tissue in the trabecular bone tissue, the major

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causes of bone necrosis are represented by a reduction in local blood perfusion and an increase in intraosseous pressure. [10]

Our research also demonstrated that HFD could cause bone microstructure and bone mass loss in mice. Mice on an HFD can have considerably improved bone microstructure after FA intervention [11, 12].

Loss of osteocytes in trabecular bone was evident in the HFD group compared to the control group, and FA mitigated these alterations. This was supported by the H&E staining of bone.

More importantly, we concluded from this result that FA might influence the development of osteonecrosis.

Homocysteine interferes with the development of intermolecular, according to a previous study that showed folic acid treatment can lower serum homocysteine levels and affect how quickly bones are broken down. cross-links that aid in stabilizing the collagen macromolecule for anomalies in homocystinlar networks in connective tissue (13)

With indications of decreased collagen cross-linking in homocystin-uria patients, later research confirmed this theory. (14). The idea that homocysteine is involved in the development of osteonecrosis is supported by studies of genetic association.

It is possible that aberrant plasma homocysteine concentrations could occur from the methylenetetrahydro-folate reductase (MTHFR) gene's reduced activity, which can interfere with the methylation of homocysteine to methionine. Elevated plasma homocysteine levels in patients with low plasma folate concentrations are linked to a frequent mutation in the MTHFR gene. (15)

It's been hypothesized that high cholesterol prevents bone production by inhibiting osteoblast differentiation. Additionally, increased osteoclastogenesis might be at play [8].

Low HDL-C concentrations have been linked to the development of an inflammatory microenvironment and increased bone marrow adiposity, which inhibit the differentiation and function of osteoblasts and result in decreased bone mass [16]. Additionally, a differential effect of serum cholesterol on bone has been proposed.

Increased lipids build up in the perivascular area and vascular intima of bones, similar to atherosclerosis [17]. Additionally, bone loss is brought on by inflammatory bioactive lipids that support atherosclerosis [17].

Other mechanisms include the buildup of fat in the bones, which causes ischemia and hypoxia by reducing bone vascularization and raising the pressure in the bone marrow microcirculation [18].

Additionally, the blood flow to the bones is compromised by increased blood viscosity. Bone necrosis could be caused by any of these occurrences [18].

According to earlier research, phospholipids, cholesterol, and fatty acids, which are crucial for bone cell survival and function, bone mineralization, and important signaling pathways, are distributed in bone marrow and other mineralized tissues and may play a significant role in regulating the physiological functions of bone.

Consequently, they can be regarded as crucial controllers of bone homeostasis. Fatty acids, on the other hand, can harm bone health and have toxic effects [19].

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