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Macrophage response in patients diagnosed with aseptic necrosis of the femoral head presenting different risk factors

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Abstract

Aseptic necrosis of the femoral head is a condition caused by partial or total interruption of blood supply to the femoral head. The diminished blood supply causes necrosis of the cellular elements and of the bone marrow, followed by the collapse of the bone structure, events that ultimately lead to the destruction of the bone tissue, the appearance of local pain and loss of function in the affected coxofemoral joint. The importance of this condition is that it mainly affects young adults aged 30–50 years, active from a socio-professional standpoint, and increased life expectancy. The material studied to achieve CD68 immunostaining was represented by bone fragments from the area of necrosis and from the adjacent areas of the femoral heads, harvested from 39 patients when performing hip arthroplasty surgery. The patients were diagnosed with aseptic necrosis of the femoral head and hospitalized in the Clinic of Orthopedics and Traumatology, Emergency County Hospital of Craiova, Romania, from June 2014 to January 2015. The 39 patients included in the study were divided into four categories according to presented risk factors (alcohol, alcohol and smoking, trauma, corticosteroids). All the 39 cases had positive immunostaining for CD68, macrophage being highlighted both in the area of necrosis and in the adjacent areas. We noted significant differences in the number and arrangement of macrophages in patients presenting different risk factors. The highest number of macrophages was present in patients presenting a risk factor corticosteroids, and the lowest number of macrophages was found in patients who had trauma as the main risk factor.

Keywords: macrophages, osteonecrosis, femoral head.

Introduction

Currently, the aseptic necrosis of the femoral head is a common cause of developing musculoskeletal disability, found among the young, with both diagnoses and treatment problems [1].

Concrete data on the incidence and prevalence of this disease are poorly known in our country, but it is known that in recent years there has been an increase in hip replacements in patients diagnosed with this condition. Internationally, in the last 20 years the incidence of this disease has significantly regrown on the one hand due to an increase in drinking, smoking, patients who received treatment with corticosteroids, patients who have undergone organ transplants and partly because of the widespread use of new diagnostic imaging techniques and increased interest for this condition. Trauma is the most common cause of developing secondary aseptic necrosis of the femoral head [1, 2], followed by alcohol and corticosteroids [1].

Aseptic necrosis of femoral head mainly affects young adults, who are between the 3rd and 5th decade of life [1–6]. It is believed that aseptic necrosis of femoral head affects men four times more than women [1, 2, 5, 6].

Aseptic necrosis of femoral head shows no clinically characteristics. In the early stages of the disease, patients are usually asymptomatic. In most patients, clinical signs of the disease can be detected a few years after its occurrence [1–3, 7].

Imaging investigations are the basis upon which diagnosis of this condition is achieved. Imaging tools used in the diagnosis of aseptic necrosis of the femoral head are plain X-ray in various incidences, magnetic resonance imaging (MRI), computed tomography (CT), bone scan, computed tomography single-photon emission (SPECT) [8].

MRI is a non-invasive imaging technique with the highest degree of specificity and sensitivity used in the diagnosis of aseptic necrosis of the femoral head [1, 2, 5, 8, 9]. In the early stages is more effective than simple CT or SPECT, with a degree of increased specificity in the detection of the condition of up to 90% [8, 10].

Materials and Methods

The studied group was represented by a total of 39 patients diagnosed with aseptic necrosis of the femoral head, stages III and IV (Liver and Arlet classification), admitted to the Clinic of Orthopedics and Traumatology, Emergency County Hospital of Craiova, Romania, during June 2014–January 2015 investigated with non-invasive imaging techniques (simple X-ray, MRI, CT), after which they underwent hip arthroplasty surgery.

Data regarding age, gender, origin, previous medical history, risk factors, the onset of clinical symptoms, duration of symptoms until presenting for a checkup, evolutionary stage of the disease, clinical diagnosis, imaging diagnosis, and number of days of hospitalization necessary after performing surgery were obtained from patient charts.

Note that this study has obtained approval from the Ethics Committee of the University of Medicine and Pharmacy of Craiova. Each patient signed an informed consent, they were previously handed a form in which they were presented all the information related to the participation in the study, further use of biological material collected during surgery and use of personal data. Clinical data and the collection of biological material were achieved after obtaining written informed consent from the patients.

The biological material used in the study was bone tissue form the necrosis area and adjacent ones, harvested from the head and neck of the femur of the 39 patients, collected when performing hip arthroplasty. Biological material was initially cut into thin longitudinal slices, which were photographed for macroscopic imaging, in order to do a correlation between macro and microscopic appearance of the sections analyzed.

The biological material was fixed in formalin, decalcified in trichloroacetic acid solution, and finally embedded in paraffin, stained with conventional histological methods, Hematoxylin–Eosin and trichrome based (Goldner–Szekely).

To highlight immunohistochemical tissue antigens a two-staged method was used based on a polymer network visualization system (Dako EnVision). Immunohistochemistry technique itself consisted of a standard algorithm, with some variation depending on the antibodies used. The antibody used for immunohistochemical study to highlight the macrophage reaction was represented by CD68 (monoclonal antibody, clone KP1, 1:50 dilution, Dako manufacturer).

Results

The 39 patients included in the study, with ages between 23 and 58 years and a gender ratio of 3.2:1 male to female, were divided according to the main risk factors they presented into four subgroups, as follows. Subgroup I consisted of 10 patients who presented as a risk factor alcohol consumption. In subgroup II were included eight patients that had as main risk factor alcohol consumption and smoking. Subgroup III included 12 patients who had suffered physical trauma in their history and the nine patients in subgroup IV were treated with corticosteroids for variable periods (Dexamethasone or Prednisolone), which is considered the main risk factor that led to the occurrence of the disease.

In the slices from patients diagnosed with aseptic necrosis of the femoral head presenting excessive alcohol consumption as a risk factor, we noticed massive destruction of bone tissue in the area of necrosis, having been replaced by fibrous tissue. The revealed fibrous tissue does not show homogeneous appearance, being different from one area to another. We noticed many areas macrophage-type cells in the fibrous, but also areas where their number was reduced or where missing. In the fibrous tissue numerous adipocytes are disseminated, most of which with hypertrophied appearance. In areas with high density of adipocytes, we noticed the absence of macrophages. Macrophages can be highlighted in small number also in the transition zone between fat and fibrous tissue. In some areas, the fibrous tissue looks uneven with collagen fibers in a messy arrangement, sometimes chaotic. At this level, a large number of macrophages are observed. In some areas, we observed the appearance of dense fibrous tissue with collagen fibers with similar sizes and shapes arranged in ordered bundles. In these areas, macrophage cells are rarely present or absent. We noticed that as the fibrous tissue becomes more organized, macrophage-type cells disappear from the lesion area (Figures 1–3).

On slides from patients diagnosed with aseptic necrosis of the femoral head presenting prolonged consumption of alcohol and smoking as a risk factor, we observed massive destruction of bone tissue in the area of necrosis and it being replaced by fibrous tissue. In these patients, we observed a small number of macrophages cells, disseminated throughout the fibrous tissue. Fibrous tissue, observed on slides from patients presenting alcohol consumption and smoking as a risk factors, is well organized, rich in collagen fibers with rare disseminated adipocytes. In some areas of fibrous tissue, adipocytes are absent. In areas with dense fibrous tissue, we noticed a very small number of macrophages or their absence (Figures 4 and 5).

In patients presenting trauma as a risk factor, we noticed besides the destruction of the bone tissue, large areas of erosion in the cartilage tissue adjacent to the necrosis area. In these patients, we observed a small number of macrophages both in the fibrous tissue and in the area of erosion of the cartilage overlying the area of necrosis (Figure 6).

In patients presenting corticosteroid therapy as a risk factor, we observed bone modification and narrowing of the trabeculae, widening of areolar cavities bounded by them, and normal tissue being replaced with fibrous tissue and the presence of adipocytes at this level. In the areolar cavities, we noted the presence of numerous macrophage cells. In these patients, we noted a large number of macrophages disseminated throughout the fibrous tissue, whether the collagen fibers were orderly layered or disorganized. We noticed, however, areolar cavities containing extended fibrous tissue with no macrophage cells present (Figure 7).

In patients who presented corticosteroids as the main risk factor, we noticed besides the presence of numerous macrophages in the fibrous tissue their particular provision, in large groups, around endosteum. On some slices, we have noted the presence of numerous blood vessels with sclerotic appearance and mainly perivascular disposition of macrophages in the fibrous tissue. We noticed, however, a decrease in the number of macrophage cells, near blood vessels in certain areas. We highlighted in most of these patients massive erosion of the cartilage tissue overlaying the area of necrosis. In the area of cartilage erosion, we noted the presence of numerous macrophages, showing intense lysosomal activity (Figures 8 and 9).

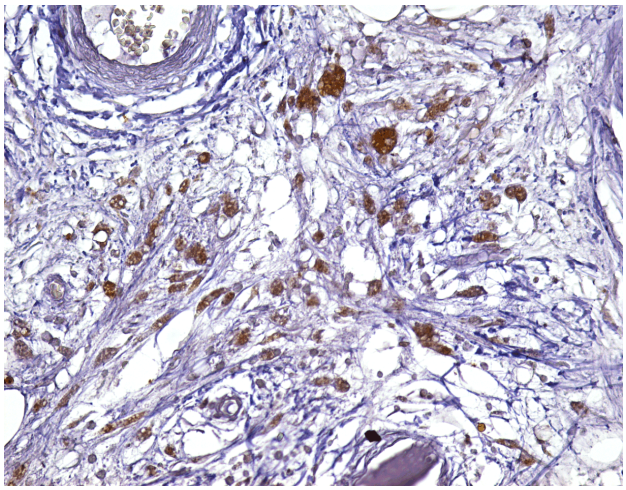

Figure 1 – ***Overview of fibrous tissue, an area with chaotic collagen bundles, macrophages of different dimensions. CD68 immunostaining, ×100.***

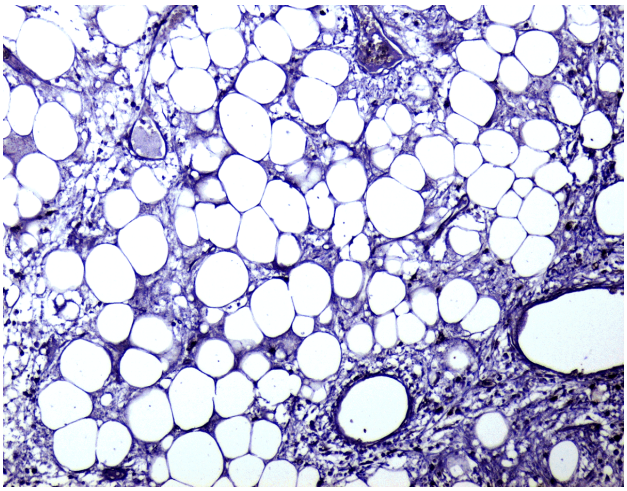

Figure 2 – ***An area occupied predominantly by fat cells, it can be noticed the absence of macrophages. CD68 immunostaining, ×100.***

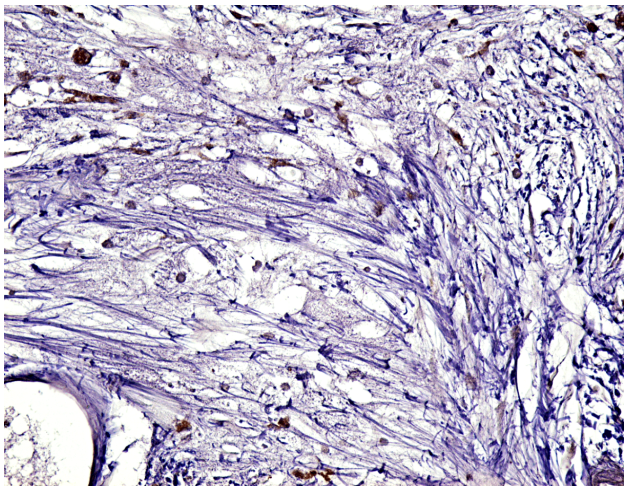

Figure 3 – ***A detail from the dense fibrous tissue areas rich in collagen fibers organized in bundles, rare macrophage-type cells. CD68 immunostaining, ×200.***

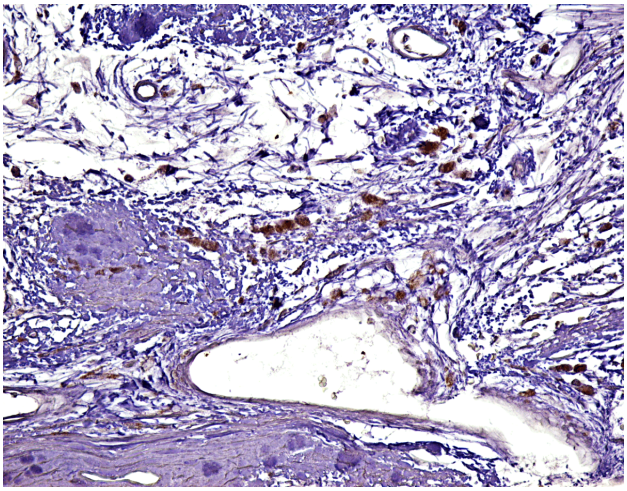

Figure 4 – ***Detail from an area of fibrous tissue, rare macrophage-type cells. CD68 immunostaining, ×200.***

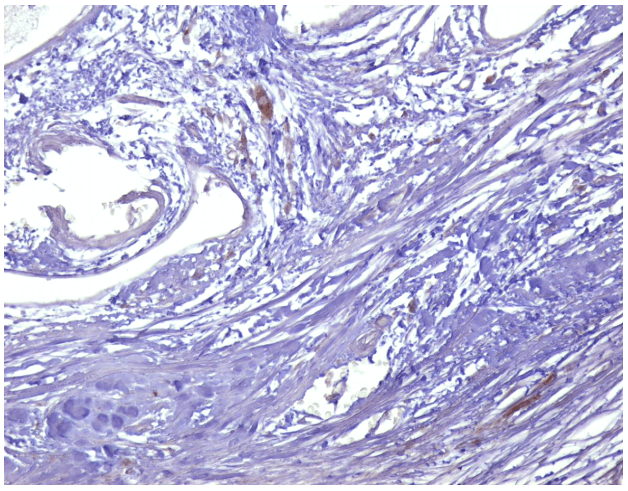

Figure 5 – ***Well-organized area of fibrous tissue rich in collagen fibers, it is noted the presence of a single macrophage cell. CD68 immunostaining, ×200.***

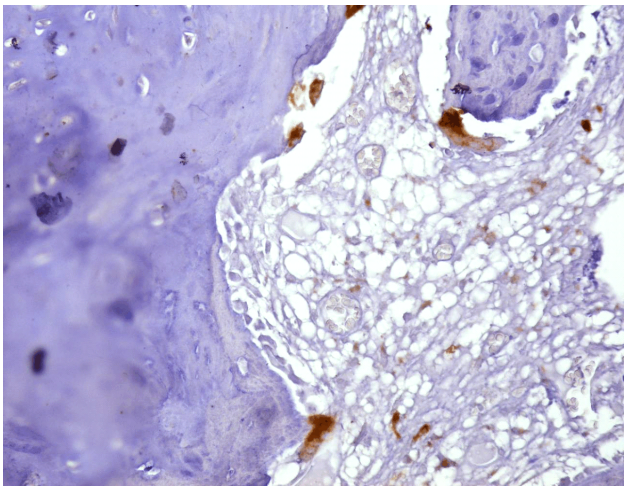

Figure 6 – ***Few macrophage-type cells located in the area of erosion of the cartilage. CD68 immunostaining, ×200.***

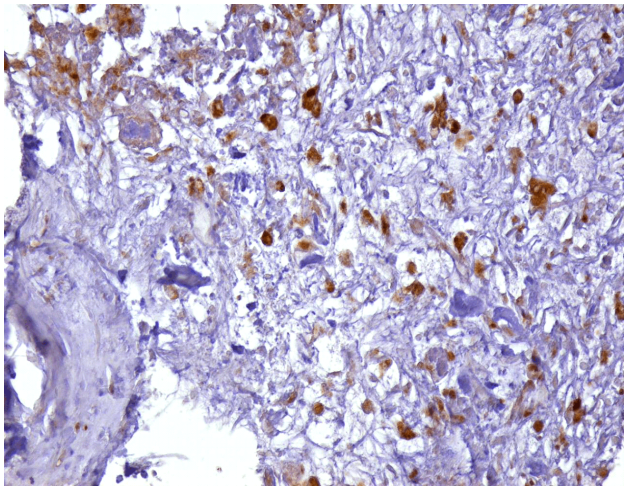

Figure 7 – ***Macrophage-type cells are unevenly distributed in the area of fibrous tissue. CD68 immunostaining, ×200.***

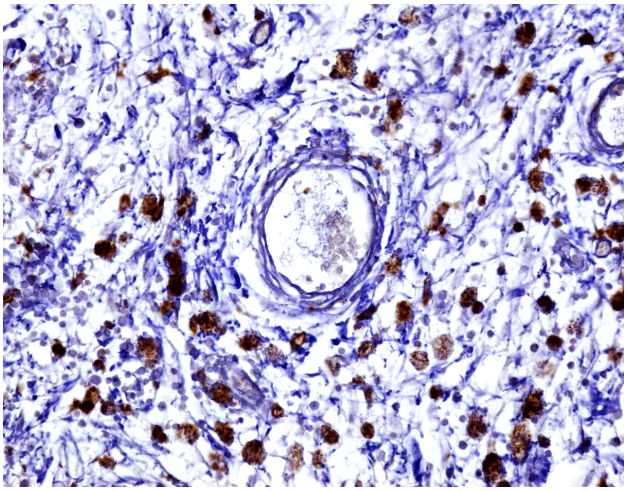

Figure 8 – ***Dense fibrous tissue, collagen fibers organized in bundles, numerous macrophage-type cells with perivascular disposition. CD68 immunostaining, ×400.***

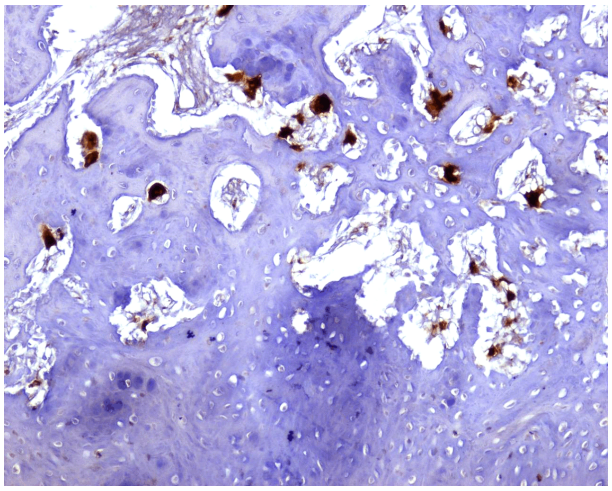

Figure 9 – ***Image overview, area of cartilage erosion, numerous macrophages. CD68 immunostaining, ×40.***

Discussion

All 39 cases had positive immunostaining for CD68, macrophage cells being highlighted both in the area of necrosis and in the adjacent areas, corresponding to the data found in the literature. A recent study revealed the presence of macrophages in patients diagnosed with aseptic necrosis of the femoral head in the bone tissue and the synovial tissue [11].

Another study has demonstrated the presence of macrophages, in the area of necrosis that occurs in some patients, in the contact area between the metal hip prosthesis and the bone it is implanted in [12].

In patients presenting a risk factor like corticosteroids (Dexamethasone, Prednisolone), we observed bone modification and bay narrowing, widening of areolar cavities defined therein, the replacement of normal tissue with fibrous tissue and the presence of excess adipocytes at this level, especially of the hypertrophied appearance.

Several hypotheses regarding aseptic necrosis of femoral head induced by glucocorticoids is based on pathophysiological mechanisms such as small vessel occlusion due to fat emboli and the decrease in blood flow to the capillary due to increased intraosseous pressure caused by fat cell infiltrate determined by corticosteroid therapy. There were many changes of lipid metabolism observed in patients who received long-term corticosteroid therapy [13, 14].

Some studies have shown that Dexamethasone administered for different time intervals is likely to induce differentiation of the stem cells of the bone marrow into adipocytes and inhibiting the process of bone formation [15–18]. Adipocyte hypertrophy was noted in patients undergoing treatment with Dexamethasone even after five days [19]. Dexamethasone has been shown to inhibit the expression of type I collagen and osteocalcin, suppression of stem cell differentiation from bone marrow into osteoblasts [15].

Prednisolone therapy has a proven role in reducing bone density and reduction of trabecular bone [20]. Mesenchymal stem cells taken from patients diagnosed with aseptic necrosis of femoral head induced by glucocorticoids have a low proliferative activity, which may explain the reduced capacity of bone matrix regeneration in these patients [21].

Treatment with lipid lowering agents can counteract the inhibitory effect of the steroid on osteoblast gene expression [22]. These findings have been demonstrated both *in vitro* and *in vivo* [22, 23]. It was found that aseptic necrosis of femoral head induced by glucocorticoids can be explained due to increased intraosseous pressure due to excessive accumulation of adipocytes in the bone marrow or a change in the differentiation of stem cells from the bone tissue excess into adipocytes, mechanisms that contribute to reducing the number of stem cells available for the production of osteoblasts, and ultimately lead to inadequate bone repair and remodeling of necrotic areas.

In samples from patients diagnosed with aseptic necrosis of the femoral head who presented a risk factor like excessive alcohol consumption, we noticed massive bone tissue destruction in the area of necrosis of and its replacement by fibrous tissue. Within the fibrous tissue there are disseminated numerous adipocytes, most of them hypertrophied.

Alcohol induces a significant increase in the level of triglycerides and cholesterol, fatty infiltration of the bone

marrow and liver. In the subchondral femoral head area in patients diagnosed with aseptic necrosis of the femoral head, we observed proliferation and hypertrophy of adipocytes. Triglyceride storage in the osteocytes lead to pyknosis and increased growth rate of areolar cavities without osteocytes.

Several studies have shown that alcohol plays an important role in proliferation and adipocyte hypertrophy, fatty degeneration osteocytes and bone bays modification issues by reducing the number and appearance of narrowed trabecular areas. Even if a significant proportion of patients with aseptic necrosis of the femoral head primary risk factor in the development of the disease is the alcohol consumption, producing pathophysiological mechanisms leading to disease are not yet sufficiently known, which contributes to the lack of effective therapies [24–29]. Alcohol consumption and the incidence of aseptic necrosis of the femoral head have a significant dose–effect relationship [30].

Adipocyte proliferation, decreased hematopoiesis, in excess storage of lipids in the osteocytes, bone marrow necrosis, reducing the number and size of trabecular bone are phenomena that occur from the early stages of aseptic necrosis of femoral head induced by excessive alcohol consumption and corticosteroids [28, 29, 31, 32].

The exact amount of steroids or alcohol needed to induce aseptic necrosis of femoral head is unknown. It seems that a more important risk factor than cumulative dose or duration of steroid treatment is the serum concentration of steroid use. There is a strong association between the total daily dosage and oral administration (as opposed to parenteral administration) of corticosteroids in patients with aseptic necrosis of the femoral head. Most cases occur after oral administration of high doses of steroids for more than one month, although there are rare cases, which occurred after the administration of therapy in a much shorter period [33]. Specific changes for aseptic necrosis of the femoral head were observed using nuclear magnetic resonance even at an interval of three months from the cessation of therapy with high doses of Prednisolone (4–8 weeks treatment) [34]. These changes were highlighted long before the onset of symptoms of the disease.

Multiple studies have shown that both corticosteroid therapy, and chronic alcohol consumption leads to accelerated adipocyte growth at the expense of osteoblast proliferation. Although the molecular mechanisms differ between the two substances involved in the development of the disease, the consequences such as an increase in the number of adipocytes in bone marrow cells, vascular damage and the reduction of repair capabilities finally leads to occurrence of cell death in the bone tissue. The role of a genetic predisposition that underlies the emergence of the disease has not been fully elucidated, but it could explain why some chronic alcohol consumption or some patients undergoing long-term therapy with high doses of steroids do not develop the disease [35, 36].

In patients presenting a risk factor like trauma, we noticed besides bone destruction, the emergence of large areas of erosion in the cartilage tissue adjacent to the area of necrosis. In these patients, we observed a small number of macrophages both in the fibrous tissue and in the area of erosion of the cartilage overlying the area of necrosis.

Trauma is the most common secondary cause of developing aseptic necrosis of femoral head [37]. A change in blood supply to the femoral head causes vascular necrosis. Another mechanism in the genesis of femoral head necrosis is tamponade effect. Aseptic necrosis of the femoral head may be asymptomatic for a long time, even in patients in whom femoral head collapse has already occurred [38]. The area of necrosis may contain areas with richly vascularized connective tissue. In the prevailing fibrous tissue transition area, we can find intravascular thrombi and fibrin clots. In these areas, we can observe collapsed blood vessels surrounded by fibrous tissue [38].

The samples from patients diagnosed with aseptic necrosis of the femoral head presenting prolonged consumption of alcohol and smoking as a risk factor, we observed massive destruction of the bone tissue in the area of necrosis and its replacement by fibrous tissue, with rare disseminated adipocytes at this level. In areas with dense fibrous tissue, we noticed a very small number of macrophages or their absence.

A significantly increased risk of developing the disease was found in smokers. As regards the causal link between smoking and the onset of the disease, several studies have confirmed the association between the effects of smoking as the only risk factor or associated to excessive alcohol consumption [26, 39].

Conclusions

All the 39 cases in the study group had positive immunostaining for CD68, macrophage cells being highlighted both in the area of necrosis and in the adjacent areas. The most intense macrophage response was observed in patients presenting corticosteroids as a risk factor, and the lowest number of macrophages was revealed in patients whose main risk factor was trauma. Numerous adipocytes were highlighted in patients presenting excessive alcohol consumption and corticosteroids as a major risk factor. In all patient subgroups, we noticed decrease or disappearance of macrophage-type cells as fibrous tissue from the lesion area became more organized.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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