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DIFFERENCE IN THE PERCENTAGE OF PHAGOCYTOSIS OF POLYMORPHONUCLEAR LEUKOCYTES IN PATIENTS WITH DIABETES MELLITUS TYPE I AND II WITH PERIODONTAL DISEASE

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ABSTRACT

Abstract – The purpose of this study was to determine the difference in the percentage of phagocytosis of polymorphonuclear leukocyte in patients with diabetes type I and type II with periodontal disease, recent COVID-19 pandemic that has affected millions of people in the world, it has been shown that patients with diabetes have a greater susceptibility to seriously developing the disease caused by the SARS-CoV-2 virus. **Materials and Methods:** The study consisted of 20 patients from the Periodontology Postgraduate clinic of the Faculty of Dentistry of the Autonomous University of Tamaulipas, who underwent a sample of 10 ml of heparinized blood was taken and 3 ml of Dextran 3% was added and incubated at 37 ° C for two hours in a serological bath, at the end of this time they were centrifuged at 1500 rpm for 6 minutes, the cells were washed with the GIBCO ™ DMEM culture medium 3 times for further counting and adjusted the population at 2-4 x 10 6 cells / ml. The experimental design consisted in using 200 µl of the cells in GIBCO ™ DMEM culture medium and 300 µl of a pure culture of *Staphylococcus aureus* was diluted in 0.85% saline solution, both reagents were incubated at 37 ° C for 45 minutes, then the cells were resuspended and it placed 1 drop on a slide, dried, fixed with methanol and stained each smear using the Gram technique to then count 100 cells that phagocytosed and not phagocytosed. **Results:** Control patients reported an average of 83.20 ± 5.81 phagocytic action, and diabetic patients type I and type II reported an average of 40.70 ± 9.92. Likewise, when analyzing the percentage of non-phagocytosis, the control patients presented an average of 16.80 ± 5.81 and the diabetic patients of 59.30 ± 9.92, which was statistically significant (p = 0.001), by other hand, comparing the percentage of phagocytosis among patients with diabetes type I and type II were not statistically significant, with an average

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of 40.20 ± 11.90 in patients with type I diabetes and 41.20 ± 8.90 in patients with diabetes type II (p=0.884). **Conclusions:** The percentage of phagocytosis of polymorphonuclear leukocytes was decreased in patients with diabetes type I and type II compared to the control group, with an average of 83.20% phagocytosis in the control group versus 40.70% of phagocytosis in the experimental group. There is a two-way relationship between COVID-19 and diabetes mellitus. On the one hand, people with diabetes are at higher risk of developing complications when they have COVID-19 and, on the other, SARS-CoV-2 could act as a diabetogenic agent by binding to ACE2 in the beta cells of the pancreas causing acute dysfunction and impaired glucose regulation.

Keywords: Diabetes Mellitus, Polymorphonuclear Leucocytes, Phagocytosis, Periodontal Disease.

1. INTRODUCTION

For Mexico, Type 2 Diabetes Mellitus (DM2) is proportionally eight times more frequent than in the rest of the world. A significant number of patients with diabetes are unaware of their own disease (40.2%) and of those who are aware of their disease, only a low proportion (11%) attend treatment regularly. In Mexico, the prevalence of diabetes is 11.8% in the population over 20 years of age, finding 12.6% in urban areas and 10.8% in rural areas, which gives a statistic of 5.5 million patients.

Murrah et al.[1] establish that the most frequent oral manifestations in patients with DM2 are periodontal lesions, xerostomia, prolonged healing, altered sense of taste, candidiasis infections and caries.

At the stomatological level, the most frequent pathology in diabetic patients is periodontal disease, which manifests itself with the same characteristics (gingivitis, bone loss and pocket formation) as in healthy patients.

There are some data that can guide us on the origin of this periodontitis and the frequency with which periodontal abscesses occur, among which the following stand out: the type of DM, the age of the patient and the degree of metabolic control, the most determining factor being the accumulation of dental bacterial plaque due to poor oral hygiene. Bone loss is greater depending on the degree of severity of DM.

Genuth et al[2] in a long-term study of the correlation between diabetes and periodontal disease, showed that severe periodontitis can represent an important factor for blood glucose levels to increase, so doctors should consider the periodontal condition of diabetic patients with

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difficulties in glycemic control. According to their study, periodontal disease can predispose or exacerbate diabetes.

On the other hand, caries rates in the diabetic population have been contradictory. Just as diabetic pathology can be different in young and adult populations, the same happens with patterns of dental caries. Another very common problem is oral candidiasis, which is an infection caused by fungi in the mouth and is more common in people with diabetes, including those who wear full dentures.[3-5] On January 30, 2020, the Director General of the WHO, declared COVID-19 a public health emergency of great international relevance, in accordance with the 2005 International Health Regulations.

Scientific research plays an important role in facilitating the prevention of contagion and the management of SARS-CoV-2 and the disease it causes, COVID-19, which to this day has a very high mortality rate worldwide declaring the pandemic in March 2020 by the World Health Organization. In general, people with diabetes are at higher risk of developing complications when they have COVID-19. In Italy, more than 2 thirds of deaths associated with COVID-19 are observed in diabetic patients. This relationship between diabetes and mortality was also evidenced in previous epidemics caused by other coronaviruses, such as the one that caused SARS in 2002 and the Middle East acute respiratory syndrome (MERS) in 2012.

2. MATERIALS AND METHODS

The selection of the 10 patients with diabetes mellitus and the 10 patients of the control group was carried out with the collaboration of the Periodontology postgraduate course of the Autonomous University of Tamaulipas, through a clinical history and a periodontal review, to which a clinical history and a periodontal review.

Subsequently, a blood sample was taken from each patient following the requirements of the WHO (World Health Organization) for this procedure.

Previously, two drops of 5,000 u/ml INHEPAR® PISA® heparin had been placed in each 16 x 150 test tube with a PYREX® screw cap, to which the blood sample of the patients and the control group was discharged. the blood was gently mixed with heparin to prevent coagulation, followed by 3 ml. of 3% Dextran in the 16 x 150 test tubes with PYREX® screw cap, later they were deposited using a 21 cm Pasteur pipette. PYREX® and a latex bulb the 10 ml of previously extracted blood, sliding it carefully along the walls to avoid hemolysis.

Both test tubes were incubated in the RIOS ROCHA® RIOSSA serological bath for two hours at 37°C and, after that time, the tubes were removed and the separation of the plasma rich in Leukocytes could be observed, the supernatant of each sample was aspirated, emptying it to a 13

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x 100 PYREX® test tube and, with the help of a 14 cm Pasteur pipette. PYREX®, both samples were matched to contain the same volume and thus the equivalence in weight at the time of centrifugation, these samples were taken to centrifuge at 1500 revolutions per minute (rpm) for 6 minutes in a GLOBE® model HKSC-110 centrifuge forming a "button of cells" at the bottom of each test tube, at the end of said procedure the plasma or supernatant of each of the tubes was decanted in a single intention, then the cells were slightly detached from the bottom of the tubes. test and applied 2 ml. of GIBCO™ DMEM culture medium (Dulbecco's Modified Eagle Medium).

The samples were centrifuged again at 1500 rpm for 6 minutes in the GLOBE® model HKSC-110 centrifuge, at the end, the supernatant of each test tube was decanted and 1 ml was applied. of the GIBCO™ DMEM culture medium to each sample to carry out the resuspension and counting of the cells, in the Neubauer Hausser Scientific® 0.100 mm chamber. and the Leica® Optical Microscope to count polymorphonuclear leukocytes with the help of a Manual Counter Model HT-1; 200µl. of the GIBCO™ DMEM culture medium containing 2 to 4 x106 of the adjusted cells and 300 µl of the Staphylococcus aureus suspension stock were incubated in the serological bath for 45 minutes at 37°C simulating the conditions of the human body to carry carry out phagocytosis.

Once the stipulated time had elapsed, the tubes were taken to centrifuge again for 6 minutes at 1500 rpm and then the cell button was resuspended and using a 14 cm Pyrex® Pasteur pipette and a latex bulb, each problem and control sample was distributed. on new and clean PEARL® slides, they were left to dry in the open air, fixed with methanol for 1 minute and Gram staining was performed on each of the samples.

The reading of the phagocytosis percentage was carried out with the help of a Leica® microscope, focusing the objective at 10x to visualize the field and then at 100x with immersion oil to achieve the desired field. First, 100 cells from the control were counted and immediately afterwards 100 cells from the patient were counted, and from these they were differentiated between the Polymorphonuclear Leukocytes that were or were not phagocytizing. (Fig. 1 A&B).

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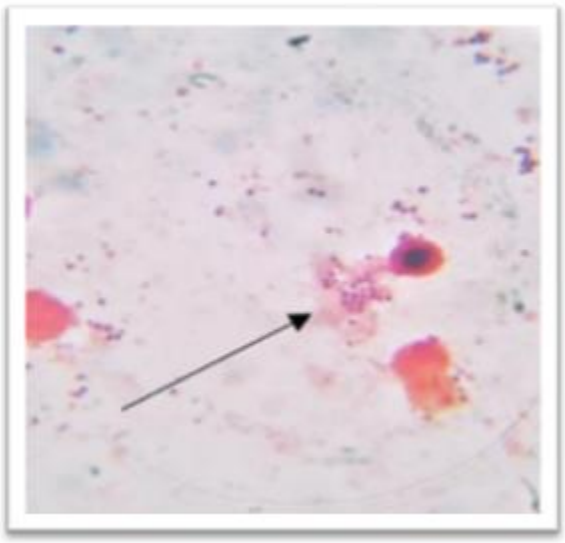

Fig. 1A: Polymorphonuclear leukocyte finishing the cycle of phagocytosis. Microscope view at 100X where Polymorphonuclear Leukocytes are observed in orange and Staphylococcus aureus in purple.

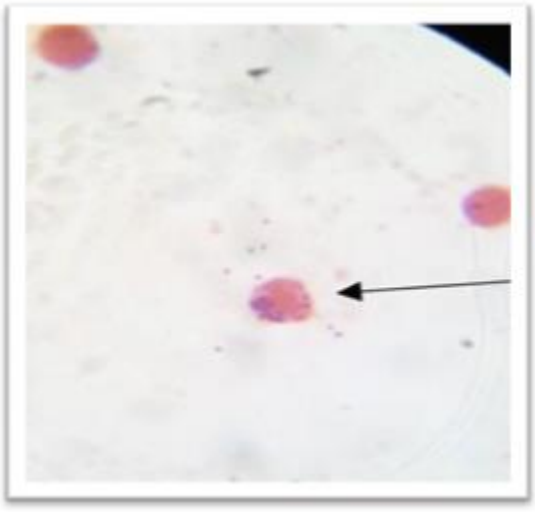

Fig. 1B: Polymorphonuclear leukocyte and inside Staphylococcus aureus (phagocytosis).

3. RESULTS

Statistical analysis began by obtaining the distribution of frequencies and percentages of periodontal status according to Russell's criteria in control and diabetic patients. In the case of the control patients, no periodontal sign was present, 100% (10) since they had healthy tissue. The opposite was the case in diabetic patients since 20% (2) presented bleeding during and after

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the probe, 40% (4) stones, 10% (1) pathological pockets and 30% (3) pathological pockets of 6 mm or greater. plus. Subsequently, Pearson's chi-square statistical test was performed, observing a statistically significant difference in the periodontal status of the patients under study (p= 0.001). (Fig. 2)

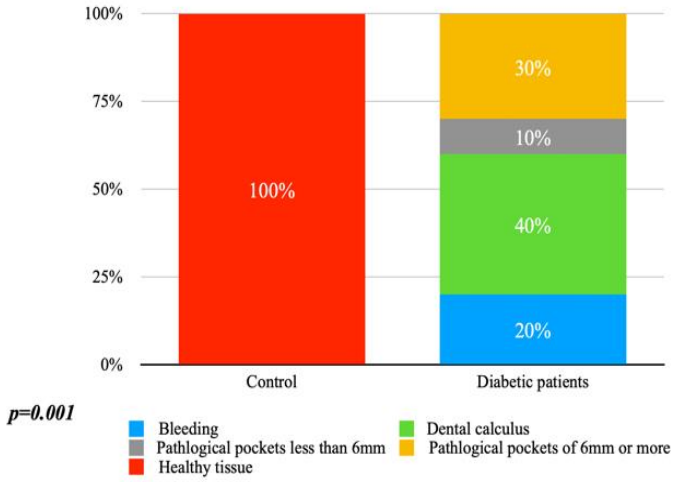

Fig. 2: Percentage distribution of the periodontal status of healthy and diabetic patients.

Likewise, the distribution of frequencies and percentages of the periodontal status of patients with Type I and II diabetes was carried out. Where it turned out that 40% (2) of type I diabetic patients manifested bleeding during and after the sounding and 60% (3) calculations. In Type II diabetic patients, it was observed that 20% (1) presented calculi, 20% (1) pathological pockets and 60% (3) pathological pockets of 6 mm or more, with no significant difference between the types of diabetes. . This was because the sample size was not enough to detect a significant association (p>0.05). (Fig. 3)

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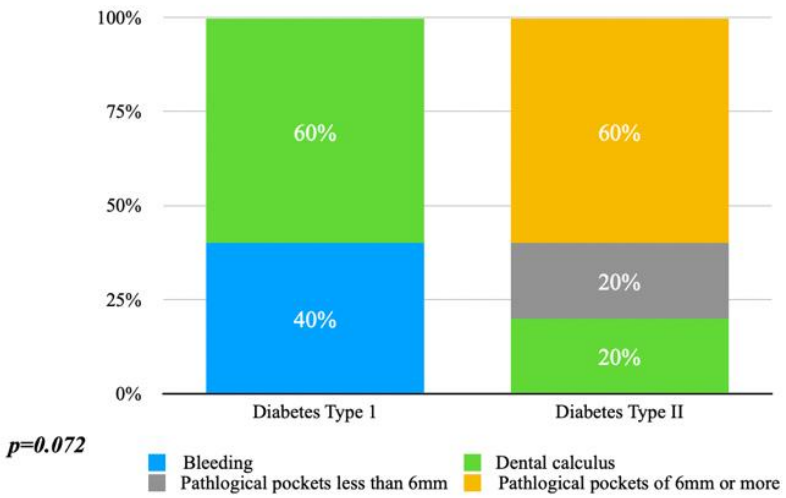

Fig. 3: Percentage distribution of periodontal status in patients with type I and II diabetes.

Percentage of Phagocytosis in Control and Diabetic patients

Continuing with the analysis, descriptive statistics were performed to evaluate the percentage of phagocytosis and non-phagocytosis in the two study groups. Healthy patients reported a mean of 83.20 ±5.81 phagocytosis and diabetic patients a mean of 40.70 ±9.92. Likewise, when analyzing the percentage of non-phagocytosis, healthy patients presented a mean of 16.80 ±5.81 and diabetics 59.30 ±9.92.

Therefore, when using the independent samples t test, it was concluded that the difference presented in the percentages of phagocytosis and non-phagocytosis between both groups is statistically significant ($p<0.05$). (Fig.4)

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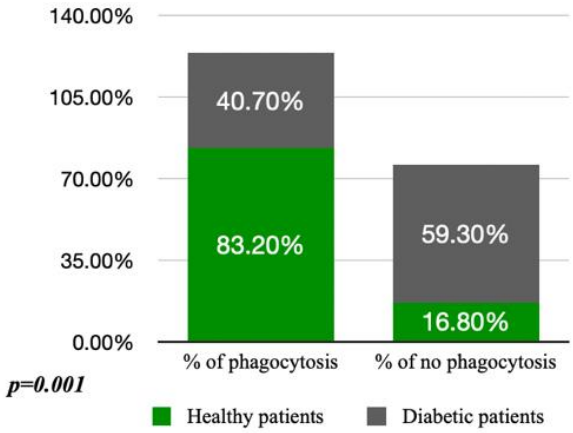

Fig. 4: Mean of the phagocytosis and non-phagocytosis variable in the control and experimental groups.

4. DISCUSSION

Since 2000, Diabetes Mellitus in Mexico is the leading cause of death among women and the second among men. In 2010, this disease caused nearly 83,000 deaths in the country.

For this reason, it was of our interest to study it in relation to periodontal disease and its interrelation with a very important parameter of the non-specific cellular immune response, which is phagocytosis.

According to the results found, diabetic patients presented a higher percentage in the values of bleeding, stones and pathological pockets after the probe, finding a statistically significant difference (p=0.001).

Mealey and Oates[6,7] reported that when they classified patients according to glycemic control, gingival bleeding was seen to be significantly higher with poor glycemic control.

Regarding the distribution of frequencies and percentages of periodontal status with type I and type II diabetes, although there was no significant difference (p=0.072), patients with type II diabetes presented: bleeding, calculus and periodontal pockets less than 6 mm., and periodontal pockets greater than 6 mm. unlike patients with type I diabetes who only manifested bleeding and calculus.

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Cutler et al[8] have pointed out that greater gingival inflammation has been seen in patients with type II diabetes than non-diabetic controls and higher levels of inflammation have also been observed in subjects with poor glycemic control.

Regarding significant difference (p=0.001) was found in terms of phagocytosis in both healthy patients versus diabetic patients, as has been pointed out by Molenaar et al and Bagdade et al[9,10].

Perillie et al[11] have shown that correction of diabetic ketoacidosis resulted in an improvement in phagocyte migration, on the other hand, Bybee and Rogers12 have shown that defects in functional phagocytosis were not confined to ketoacidosis, but it also occurs in poorly treated diabetic patients.

For all of the above, we postulate that from phagocytic cells such as LPMs, both eosinophils and neutrophils, as well as the monocyte/macrophage system, when there is a drop in leukocyte phagocytosis, a leukocytosis occurs as a feedback Monnier et al[13], as well as a hyperresponse of the monocyte/macrophage complex that results in a significant increase in the production of mediators and pro-inflammatory cytokines, it is known that the products of glucose, lysine and arginine residues (AGEs) (Advance glycation end products) have multiple effects on cell-cell and cell-matrix interactions, being closely related to the complications of diabetics since high levels of complex-periodontal-AGEs accumulation are found in such subjects with diabetes.

These AGEs activate their receptor known as RAGE (Receptor for AGE) found on the surface of smooth muscle, endothelial cells, neurons, and the monocyte/macrophage complex[14,15].

It is important to recognize that hyperglycemia results in increased expression of RAGE, and that AGE-RAGE interaction on the endothelium causes increased vascular permeability and thrombus formation. On the other hand, AGE/RAGE interaction on monocytes increases cellular stress. oxidative stress and activates the transcription of nuclear factor kappa β (NF-kB), which alters the phenotype of the monocyte/macrophage complex, resulting in increased production of pro-inflammatory cytokines such as interleukin (IL-1β), tumor necrosis factor (TNFα) and interleukin.[16]

These pro-inflammatory cytokines contribute to the pathogenesis of periodontal disease and probably play a major role in diabetic patients, especially when glycemic control is poor, as was the case in our patients.

Since the hyperglycemic state includes inhibition of osteoblastic cell proliferation and collagen production which in turn results in reduced bone formation since it acts by decreasing the mechanical properties of new bone formation.

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Finally, there is additional emerging evidence that the decrease in matrix-producing cells, critical for the maintenance of the periodontium, including fibroblasts and osteoblasts, occurs due to an increased rate of apoptosis in a hyperglycemic state in response to Porphyromonas gingivalis.[16]

5. CONCLUSION

1. The percentage of phagocytosis observed in control patients was 83.20%.
2. The percentage of phagocytosis of type I diabetic patients with periodontal disease was 40.20%.
3. The percentage of phagocytosis of type II diabetic patients with periodontal disease was 41.20%.
4. The percentage of phagocytosis between control patients and patients with type I and type II diabetes was significant. (p=0.001)

There is a bidirectional relationship between COVID-19 and diabetes mellitus. On the one hand, people with diabetes are at higher risk of developing complications when they have COVID-19 and, on the other hand, SARS-CoV-2 could act as a diabetogenic agent by binding to ACE2 in the beta cells of the pancreas, causing acute dysfunction and impaired glucose regulation. To date, there are no clear data on the impact of this pandemic on the incidence of chronic complications associated with diabetes; however, it is essential to optimize the metabolic management of patients in order to improve prognosis and reduce the burden on health systems.

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