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Biocompatibility Study Concerning Hematological Reactions Accompanying Subcutaneous Implantation of Some Dental Products in Wistar Rats*

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Abstract. The experiment aims to evaluate, by a hematological point of view, the consequences (biocompatibility) of subcutaneous implantation of some dental composites (Ivoclar, Noritake, Vita, Vitadur) special prepared for this purpose, on blood cells (RBC, WBC and PLT). The experiment was conducted on 45 healthy Wistar adult rats, randomly and equally distributed in 9 groups of 5 animals per group. The implantation of dental composites was made subcutaneously, in the dorso-thoracic region of the rats. Subsequently, the rats were observed during the experiment (10 weeks) for any clinical (local or general), pathological and hematological reactions. The results obtained did not show any clinical or pathological effects, neither locally or even less systemic. As for hematological parameters investigated, the values obtained in experimental groups comparatively with the reference one did not show, with a very few exceptions, statistically significant differences, which indicate a possible pathogen effect of the studied dental composites on the bone marrow hematopoietic centers, or on the cellular components of blood, whether it's red, white blood cells or platelets.

Key words: Wistar rats, hematology, Ivoclar, Noritake, Vita, Vitadur.

INTRODUCTION

The toxic potential of dental ceramics is considered negligible due to their excellent chemical stability and their low degree of abrasion. However, the pathogen potential of ceramics can still be taken into account due to the release of a great quantity of toxic compounds in unusual circumstances. Possible pathological effects of ceramic on human and animal body may be of toxic nature, allergy, carcinogenic, teratogenic, mutagenic and that the induction of sexual sterility. All these factors reach the body by accidental ingestion of inlays, outlays or dental crowns. It must be said that the dental ceramics, as restorative material, contains many chemical substances with a great degree of toxicity, of which are important: *(Domingo et all, 1989)*

Aluminum has a relative low absorption rate, but it still can provoke kidney failure and favors the Alzheimer disease progress *(Tateishi et all., 1993).* Calcium (Ca2+), even if the most calcium compounds are relatively free of adverse effects, calcium oxide powder form can be pathogenic due to mechanical effects on the lung tissue integrity. Fluor salts: maximum admitted dose is 2 mg/m^3, and the total quantity of Fluor released by 32 crowns is estimated at 0,1 mg/day. Lithium: the toxicity appears at two mmol/L plasma concentration. Magnesium: some compounds can induce kidney failure. Titanium: except titanic acid, all its salts are safe *(Wang et all., 2003).* Besides the elements above, the dental ceramic contains certain quantities

of Na, K, Cl, Zr, Zn or other micro minerals, with no toxicity demonstrated yet. Another side effect of physical and chemical degradation of dental ceramic is the release of radioactive compounds *(Moore, 1974, Wunderlich., 1986, Veronese, 2006,)*, such as uranium oxide (UO_2) and cesium oxide (CeO_2), found in the glass (1000 ppm). The annual irradiation dose is 2,7 REM for dental crowns with uranium, value which exceeds the standard limit of 1,5 REM/year.

MATERIALS AND METHODS

The study was conducted on 45 healthy Wistar adult rats, females, randomly and equally distributed in 9 groups, each of them containing five animals: Group 1 (n=5) with an average weight of 167 ± 3,5 g/animal was considered control group (M); Group 2 (n=5) with an average weight of 203±5,2 g/animal was inoculated subcutaneous (sc) in dorso-thoracic region with a fragment of Ivoclar D; Group 3 (n=5) with an average weight of 198±4,2 g/animal was inoculated sc. with a fragment of Ivoclar DSM (enamelled); Group 4 (n=5) with an average weight of 188±3,6 g/animal was inoculated sc. with a fragment of Notitake D; Group 5 (n=5) with an average weight of 212±4.0 g/animal was inoculated sc. with a fragment of Notitake DSM; Group 6 (n=5) with an average weight of 209±3.0 g/animal was inoculated sc. with a fragment of Vita D; Group 7 (n=5) with an average weight of 193±6.0 g/animal was inoculated sc. with a fragment of Vita DSM; Group 8 (n=5) with an average weight of 206±8.0 g/animal was inoculated sc. with a fragment of Vitadur D; Group 9 (n=5) with an average weight of 196±11.0 g/animal was inoculated sc. with a fragment of Vitadur DSM. The animal groups were weighted at the beginning and the end of the experiment, in mean time they were examined for any local or general reactions. The dental materials were specially made for this experiment at the Dental Medicine Faculty Cluj-Napoca, Romania, in fragments square with sides of 4 mm and 1,5 mm height. The experiment was conducted for 10 weeks period. At the end of the experiment, the animals were etherize with ethylic ether and then killed by cardiac puncture. Were collected the blood samples on EDTA to determine the hematological parameters investigated. The measurements were made with the ABACUS junior semiautomatic device. The results were statistically processed by calculating the average (x), standard deviation (±s), percentage difference (D%) and the ``t`` test (Student).

RESULTS AND DISCUSSION

The clinical evaluation of the experimental groups did not show any local or systemic reaction to suggest a possible toxic effect of dental materials. To determine the biocompatibility of these composites, we investigated the values of some hematological parameters, as erythrocytes (RBC, HGB, HCT, MCV, MCH, MCHC, RDWs, RDWcv), leucocytes (WBC, LYM, MID, GRA) and platelets (PLT, PCT, MPV, PDWs, PDWcv) constituents. The results obtained, meaning the percentage differences between the experimental groups (inoculated with dental alloys studied) and reference group at the end of the experiment are illustrated in the following charts. The interpretation of results was made by comparing the values obtained in the experimental groups with those obtained in the reference group (RBC=7,78±0,29x10^{12}/L, WBC=7,41±0,44x10^{6}/L, PLT=538±99,61x10^{4}/L), but also to information provided by the literature in this area. *(Johnson-Delaney, 1995, Marcus, 2004, Cohn & Clifford, 2006)* Concerning to the obtained values in the control group, they are consistent with those found in the literature (RBC=6,76-9,20x10^{12}/L, HGB=11,5-16,1 g/dl, HCT=37,6-50,6 %, WBC=6,6-12,6 x 10^{6}/L, PLT=160-460 x 10^{4}/L). As the study aims to assess biocompatibility of composite materials already used in human dental

medicine, such as Ivoclar, Noritake, Vita and Vitadur, the laboratory results obtained completed less explored issues in this field: it's about the reactions recorded in the figurative elements of blood (erythrocytes, leukocytes and platelets) in Wistar rats, but also of some other parameters correlated with each of the investigated blood cell. Regarding the groups inoculated with Ivoclar D and DSM, were not found the significant percentage difference on erythrocytes constituents' determination, the values of these parameters being close to those obtained in the control group (M), and within the limits of variation of the literature. (Chart 1)

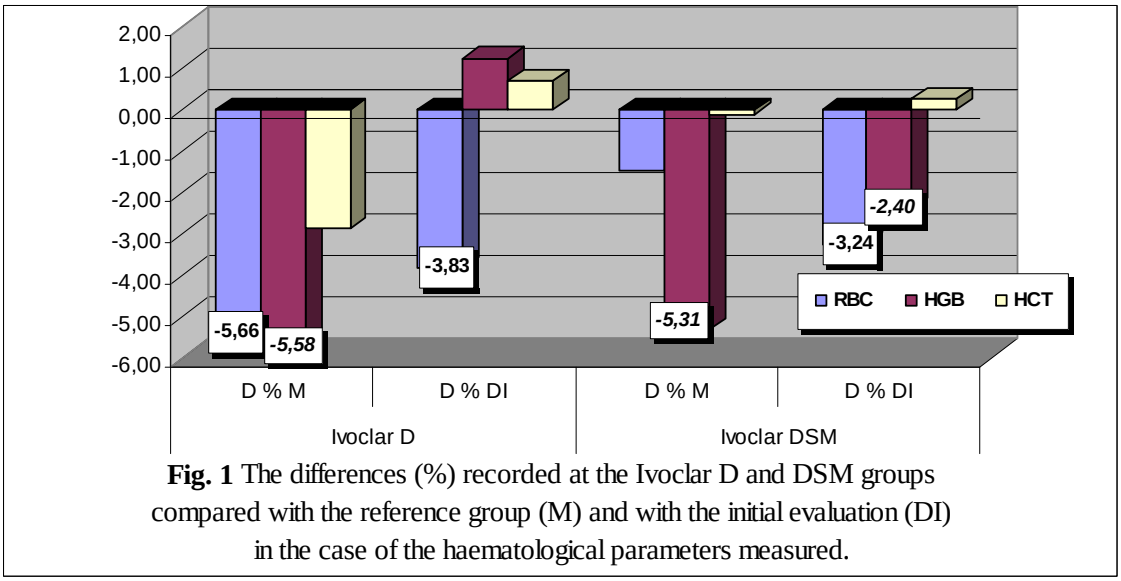

Fig. 1 The differences (%) recorded at the Ivoclar D and DSM groups compared with the reference group (M) and with the initial evaluation (DI) in the case of the haematological parameters measured.

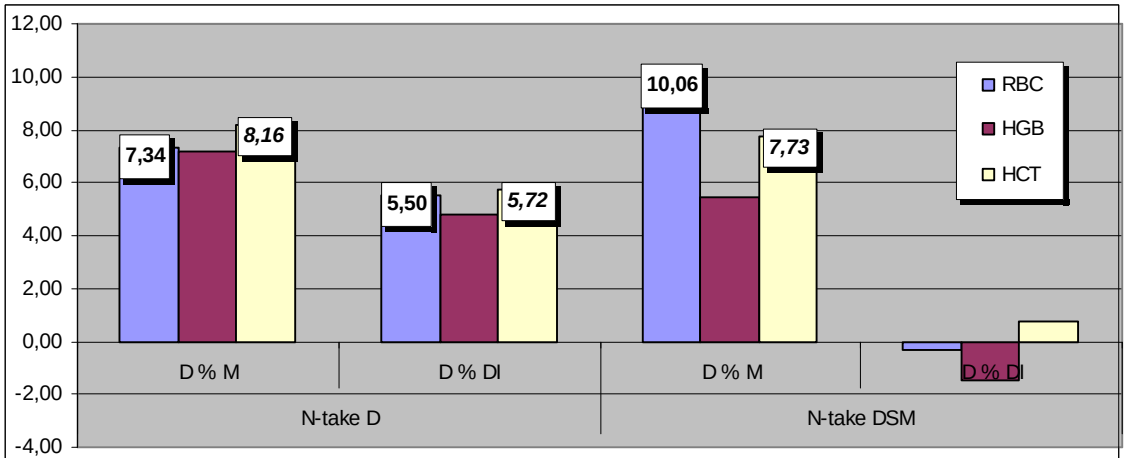

Chart 2 The percentage differences (%) recorded at the Noritake D and DSM groups comparatively with the reference group and with the initial evaluation (DI) in the case of the haematologica parameters measured

That is still available despite some positive differences that appear in both Ivoclar groups (D and DSM), because they are not exceeding the normal limits. Neither in Noritake D nor in Noritake DSM groups are any significant differences to the reference group, or to the initial determination (DI). The results had shown some positive percentage differences, but they are still situated within the normal limits. Similar aspects can be observed in the Vita D and DSM

groups. In the Vitadur D and DSM groups (Chart 4), the results express negative differences, but still in the normal limits. In conclusion, concerning the erythrocyte parameters investigated, there is no red blood cells modification regarding the organism reaction towards the dental materials we tested. This conclusion is also available for the erythrocyte investigated constituents, as MCV, MCH, MCHC, RDWs and RDWcv, whose values are close to those found in the reference group, and also to those provided by literature. *(Johnson-Delaney, 1995)*

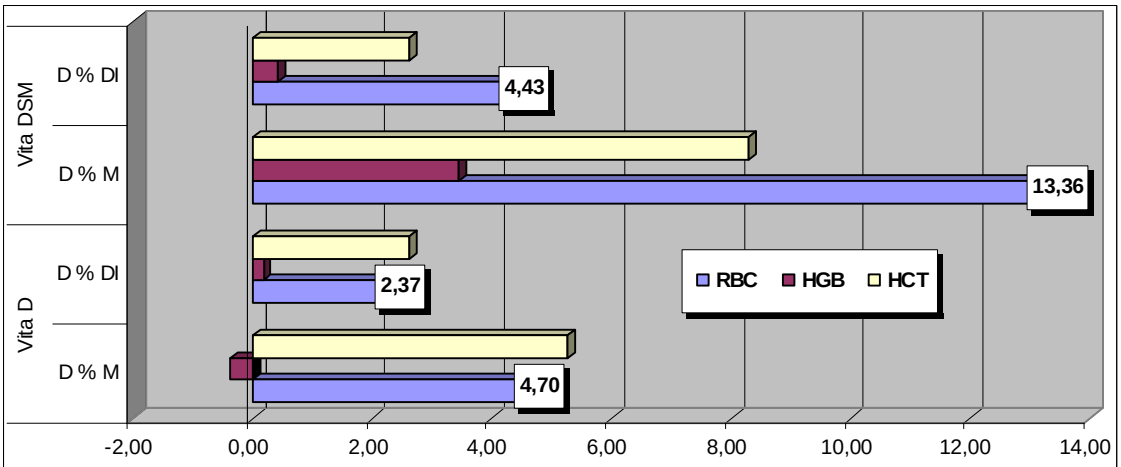

Chart 3 The percentage differences (%) recorded at the Vita D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in the case of the haematological parameters measured

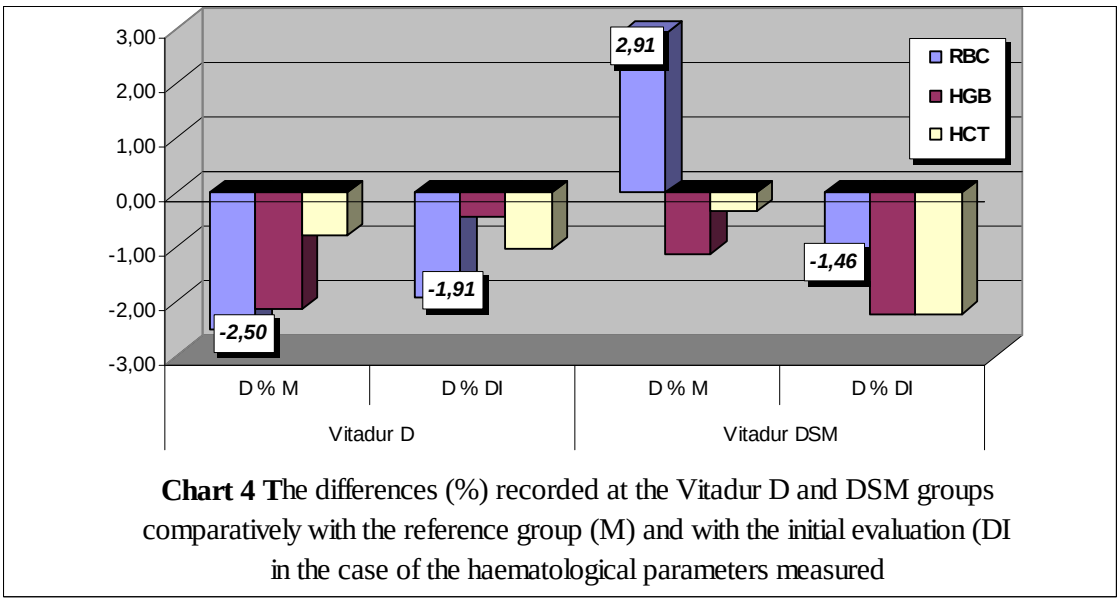

Chart 4 The differences (%) recorded at the Vitadur D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI in the case of the haematological parameters measured

Following the white blood cells determinations (WBC, LYM, MID, GRA) in the Ivoclar D and DSM groups, the results had shown a significant leucopenia reaction (4.96±1,71 x 10^6/L), resulting a percentage difference of -49,45% comparatively with the control group and of -47,93% to the initial determination (DI). This negative variation recorded is based on the decreasing of the relative and absolute value of the lymphocytes (-90.55 comparatively with the reference group and -49.45 with the initial determination).

There are no other significant changes in the values of leucocytes parameters in the experimental animals, comparatively with the control group, or compared with the data from the literature. The situation is slightly different in Noritake D group, where there is a tendency to increase leucocytes value, due to a neutrophilia, compared with the reference group (M).

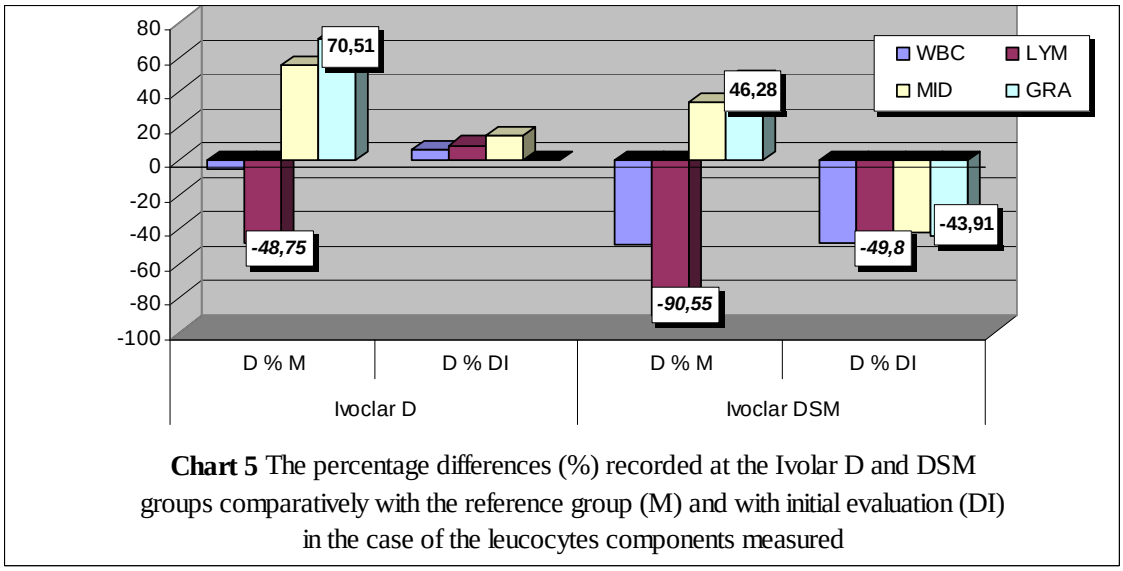

Chart 5 The percentage differences (%) recorded at the Ivolar D and DSM groups comparatively with the reference group (M) and with initial evaluation (DI) in the case of the leucocytes components measured

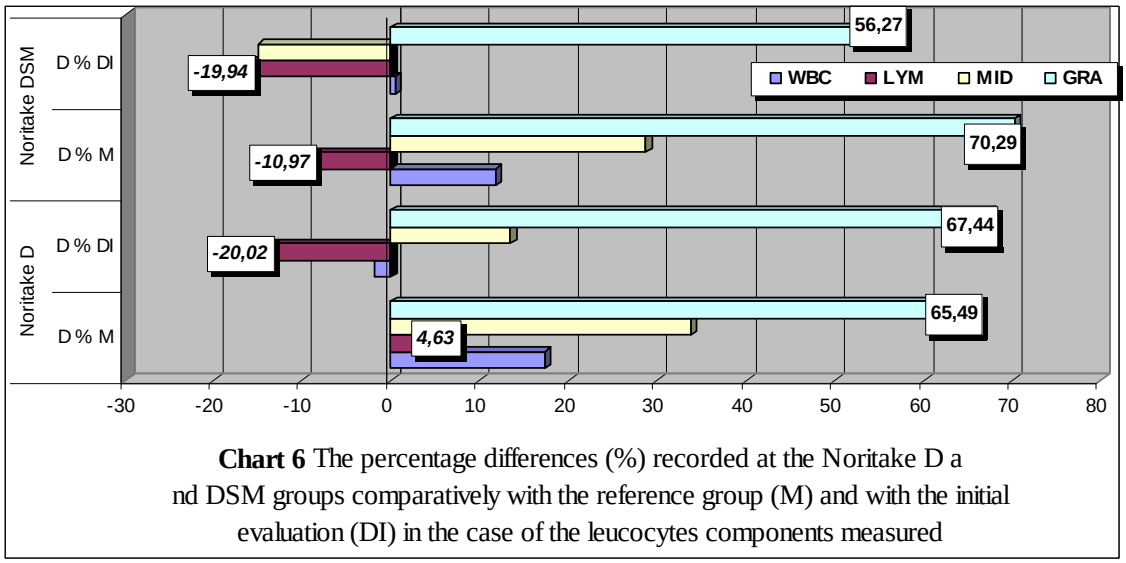

Chart 6 The percentage differences (%) recorded at the Noritake D a nd DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in the case of the leucocytes components measured

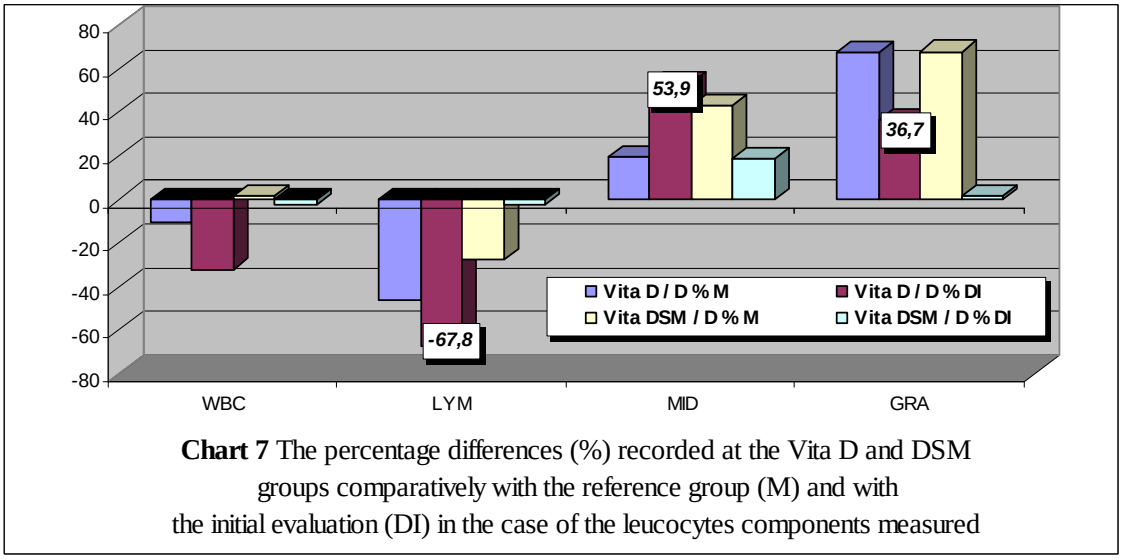

Chart 7 The percentage differences (%) recorded at the Vita D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in the case of the leucocytes components measured

This positive difference is not maintained if compared the leucocytes values with literature data, which stating the oscillation limits for total leucocytes between 6.6 and 12.6 x 10^9/L.

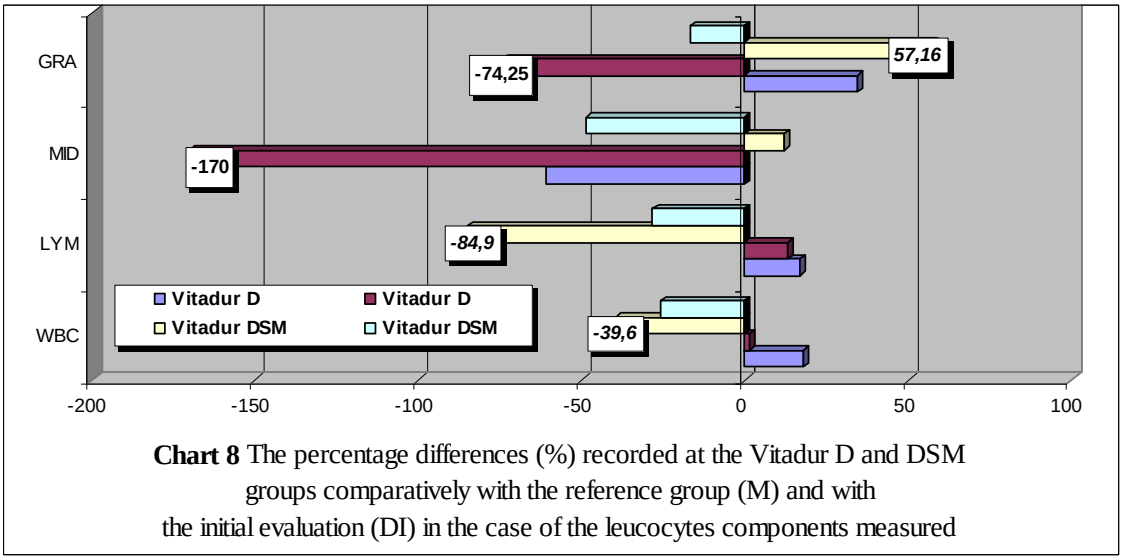

Chart 8 The percentage differences (%) recorded at the Vitadur D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in the case of the leucocytes components measured

The variations obtained in Vita D and Vita DSM groups (Chart 7) are also evasive, in which case the total leucocytes number and leukocyte formula are in normal literature limits, and very close to control group also. This fact explains those very small percentage difference recorded between the final determination values (DF), control group values (M) and the initial determination (DI) values, at least for total leukocytes number. The neutrophils show positive differences to control group in Vita D and DSM groups. The results obtained in Vitadur D and DSM groups (Chart 8) did not show any significant changes comparatively with the reference group or with the literature data, the obtained values fit perfectly within the oscillation limits considered normal for this species. On the results of the platelets parameters determinations, they have not been plotted, because there were not recorded significant percentage differences compared with the control group, with the initial determination or with the data provided from literature, to justify their detailed presentation and discussion.

The main goal of the current experiment was to evaluate, from the hematological point of view, the biocompatibility of subcutaneous implantation of some human dental materials (Ivoclar, Noritake, Vita, Vitadur) in Wistar rats. Regarding the results discussed above, one can conclude that the hematological parameters we investigated did not record significantly changes in comparison with the control group and with the data provided from literature.

CONCLUSIONS

Following the analysis and the interpretation of the results obtained in this experiment, one can be concluded that none of the dental composites (Ivoclar D and DSM, Noritake D and DSM, Vita D and DSM, Vitadur D and DSM) that had been tested produced any local and/or systemic reaction that might suggest some pathogen implication for the animals.

The determination of the hematological parameters and their constants, as red blood cells (RBC, HGB, HCT, MCV, MCH, MCHC, RDWs, RDWcv), white blood cells (WBC, LYM, MID, GRA) and platelets (PLT, PCT, MPV, PDWs, PDWcv), did not reveal any significant changes, having pathologic character, in the sense that almost all the values obtained falls within the limit of the literature, and were close to those of the reference group.

The few major changes that affected leucocytes in some experimental groups can be considered accidentals and without any biological significance, given their very small scale.

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