

Biocompatibility Study Concerning Serum Biochemical Changes that Accompany Subcutaneous Implantation of Some Dental Materials in Wistar Rats*

Florina Daiana Oșan¹⁾, D. Borzea²⁾, I. Tătaru¹⁾, Iulia Prodan³⁾, R. Mănălăchioae³⁾, I. Marcus³⁾

¹⁾Dental office, Baia Mare, B-dul. Unirii, Nr.11A, MM, *florina_osan@yahoo.com*

²⁾Faculty of Dental Medicine, University of Medicine and Pharmacy Cluj-Napoca

³⁾Faculty of Veterinary Medicine, Dept. of Pathophysiology,
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca

Abstract. The experiment is a biocompatibility study performed on Wistar rats. The goal of this experiment was to evaluate the physiological and pathological effects of some human dental materials implanted subcutaneous in Wistar rats. Were measured the serum levels of some biochemical constituents, such as enzymes (ASAT, ALAT, PAL, GGT), proteins (total serum proteins, albumins, globulins and A/G ratio) and minerals (Ca²⁺, Mg²⁺), which could provide some information on the tissues responses to implantation of these dental composites (Ivoclar, Noritake, Vita, Vitadur). The experiment was performed on 45 healthy Wistar adult rats, females, randomly and equally distributed in 9 groups, each of them containing five animals. The dental materials were implanted subcutaneously in the dorsal region of the rat's thorax. Subsequently, the animals were observed during the whole period of the experiment (10 weeks), from the clinical (local or general), pathological and serum biochemical changes point of view. The clinical evaluation didn't show any harmful effects on the animals. Concerning the biochemical parameters investigated, the variations recorded in the inoculated animals groups comparatively with the reference group didn't show, except some isolated cases, significant statistically differences, which could suggest a pathogen effect of the studied materials.

Key words: Wistar rats, serum enzymes and proteins, Ivoclar, Noritake, Vita, Vitadur.

INTRODUCTION

The toxic potential of dental composites (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 of 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 al, 1989*)

Aluminum has a relative low absorption rate, but it still can provoke kidney failure and favors the Alzheimer disease progress (*Tateishi et al., 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/m³, 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 titanitic acid, all its salts are safe (*Wang et al.*, 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 (UO₂) and cesium oxide (CeO₂), 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.

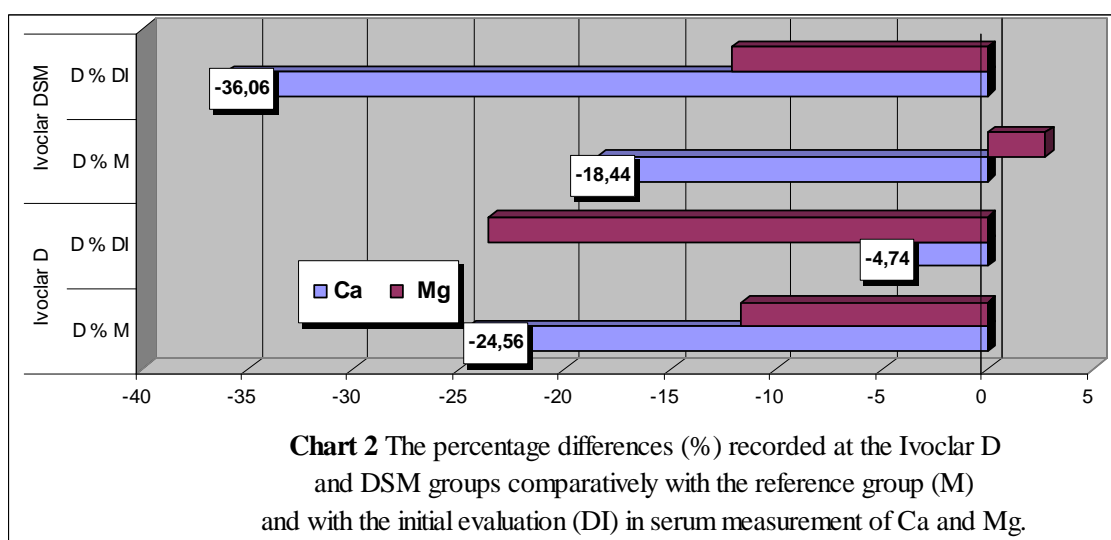
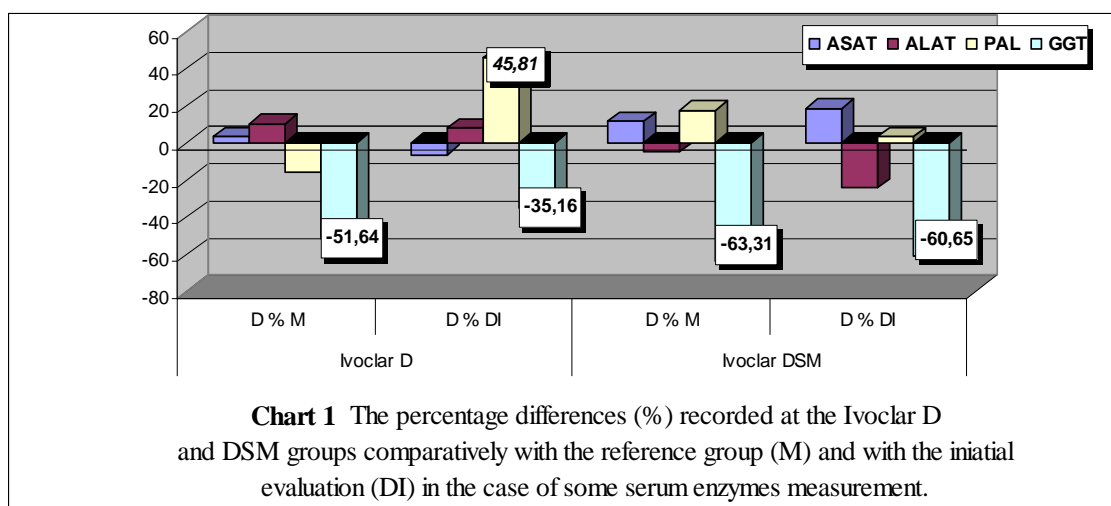
MATERIALS AND METHODS

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 $167 \pm 3,5$ g/animal was considered control group (M); Group 2 (n=5) with an average weight of $203 \pm 5,2$ 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 $198 \pm 4,2$ g/animal was inoculated sc. with a fragment of Ivoclar DSM (enamelled); Group 4 (n=5) with an average weight of $188 \pm 3,6$ g/animal was inoculated sc. with a fragment of Noritake D; Group 5 (n=5) with an average weight of $212 \pm 4,0$ g/animal was inoculated sc. with a fragment of Noritake DSM; Group 6 (n=5) with an average weight of $209 \pm 3,0$ g/animal was inoculated sc. with a fragment of Vita D; Group 7 (n=5) with an average weight of $193 \pm 6,0$ g/animal was inoculated sc. with a fragment of Vita DSM; Group 8 (n=5) with an average weight of $206 \pm 8,0$ g/animal was inoculated sc. with a fragment of Vitadur D; Group 9 (n=5) with an average weight of $196 \pm 11,0$ 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 periodically for any local or general reactions. The dental materials were specially made for this experiment at the Dental Medicine Faculty Cluj-Napoca, in fragments square with sides of 4/4 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 biochemical parameters investigated. The measurements were made with the STAT FAX 1904 semiautomatic device. The results were statistically processed by calculating the average (x), the standard deviation ($\pm s$), the percentage difference (D%) and the ``t`` test (Student).

RESULTS AND DISCUSSIONS

The clinical examination of the animals during the experimental period did not show any local and/or systemic reactions to suggest possible pathogen effects of the dental materials we tested. The evaluation of biocompatibility, from the serum biochemical point of view, was determined through measurement of the serum level of some enzymes (ASAT/GOT, ALAT/GPT, PAL and GGT), respectively of serum proteins (total proteins, Albumins, Globulins, A/G ratio) and the serum concentration of calcium and magnesium. The results obtained were interpreted by comparison to control group (M) and to the data provided by the literature (*Johnson-Delaney, 1995, Marcus, 2004, Cohn & Clifford, 2006*). Also, the results obtained at the final determination (DF) of the investigated biochemical parameters were reported in those obtained at the initial determination (DI). As for some of the enzymatic parameters investigated (ex: ASAT) the literature values showed large oscillations, depended on cited author, we use as reference values the results obtained at control group (M).

The values recorded for the biochemical parameters showed that the reference group and Ivoclar D and DSM groups fell well into physiological limits for this species of animals. This is true both for the enzymes (ex: ASAT: 45,7 – 80,8 UI/l) (Chart 1), the minerals Ca²⁺ (7,2-13,9 mg/dl) and Mg²⁺ (1,6-4,44 mg/dl) (Chart 2) and the serum proteins (PT=5,6-7,6 g/l, Alb=3,8-4,8 g/l, Glob=1,8-3,0 g/l and A/G=0,72-1,21) (Chart 3). The percentage differences which appear comparatively with the control group are within the limits of oscillation (sometimes very large) of the literature. The most significant changes affect GGT values, whose serum level decrease in the two groups, compared with both the control (-51,64% and -63,31%) and the initial evaluation (-35,16%, and -60,15%). The same situation was recorded for magnesium and calcium (-24,56% in Ivoclar D and -18,44 % in Ivoclar DSM compared with control group) serum level. On the other hand, on can observe that in the most another cases, the differences are very small and without any statistically significance. This makes very difficult the biological interpretation of the results obtained. In addition, the differences persist towards the reference group, even if the ration is made to the initial evaluation.



A similar situation is found in the Noritake D and Noritake DSM groups (Charts 4-5-6). In this case the differences recorded comparatively with the control group and with the initial determination are also relatively large for the serum enzymes (-46,37% PAL in Noritake D

compared with DI and +40,46% for the same parameter in Noritake DSM compared with control and +29,46% compared with DI), but the mean values are in normal limits. The proteins values are in the normal limits also (5,6-7,6 g/dl), both for Noritake D and DSM groups.

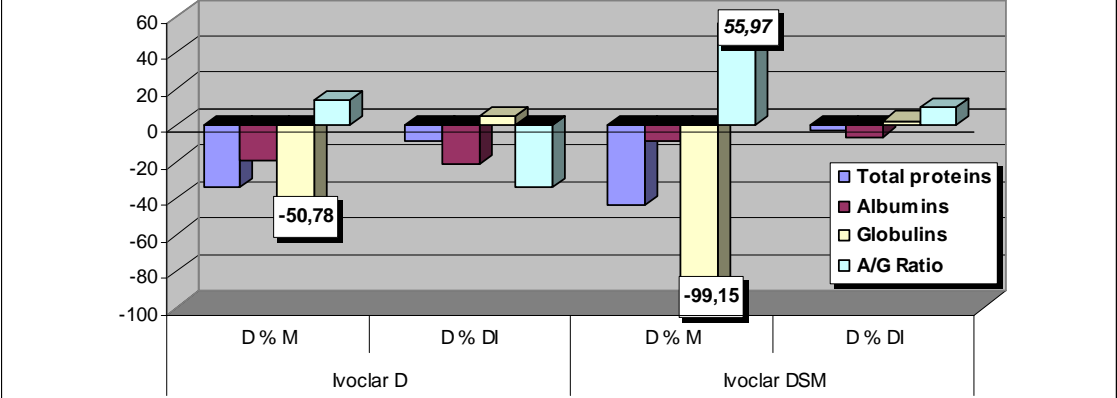


Chart 3 The percentage differences (%) recorded at the Ivoclar D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in the case of serum proteins measurement.

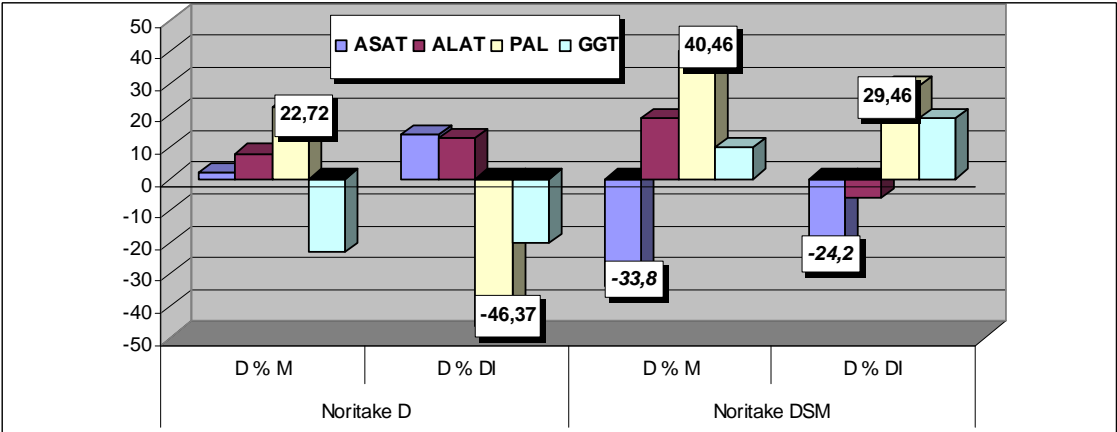


Chart 4 The percentage differences (%) recorded at the Noritake D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in some serum enzymes measurement

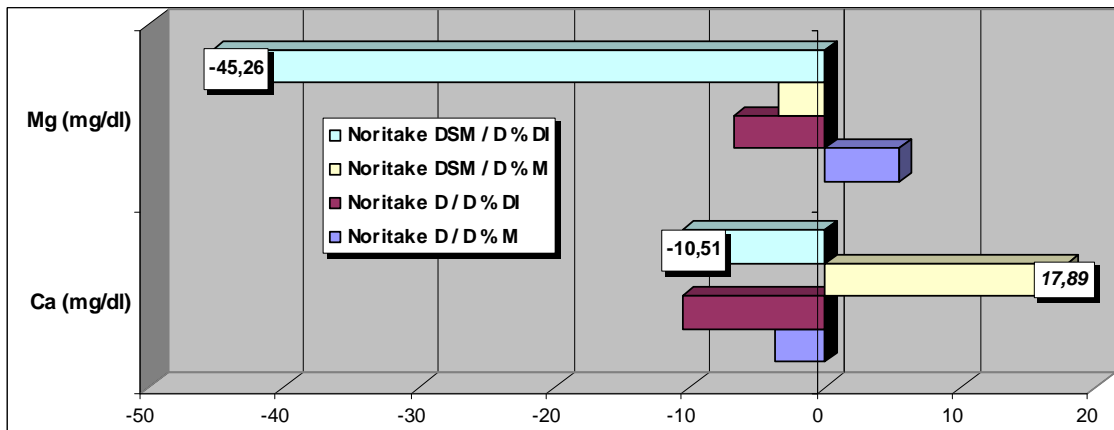


Chart 5 The percentage differences (%) recorded at Noritake D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in serum measurement of Ca and Mg

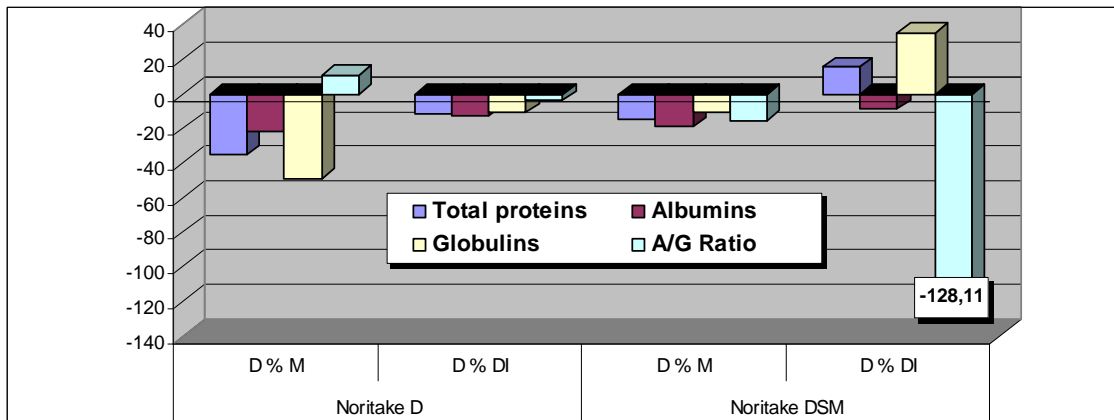


Chart 6 The percentage differences (%) recorded at the Noritake D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in serum proteins measurement.

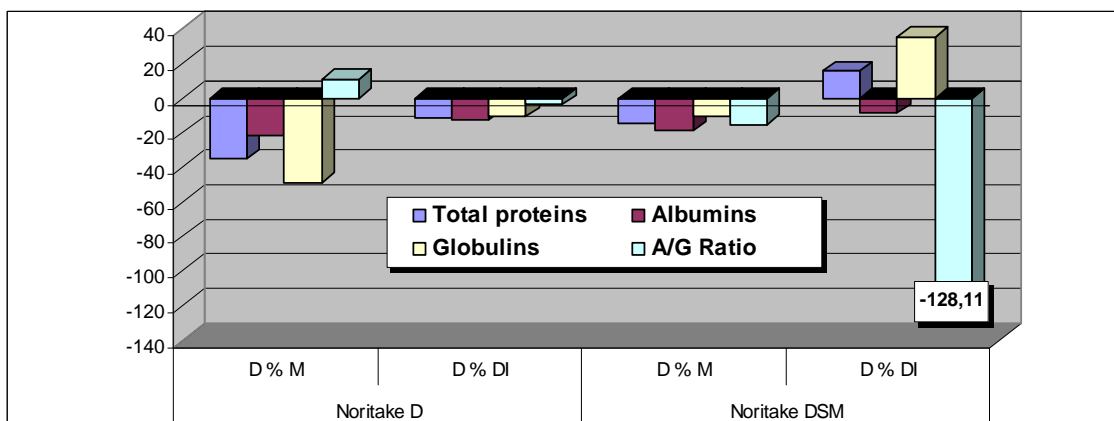
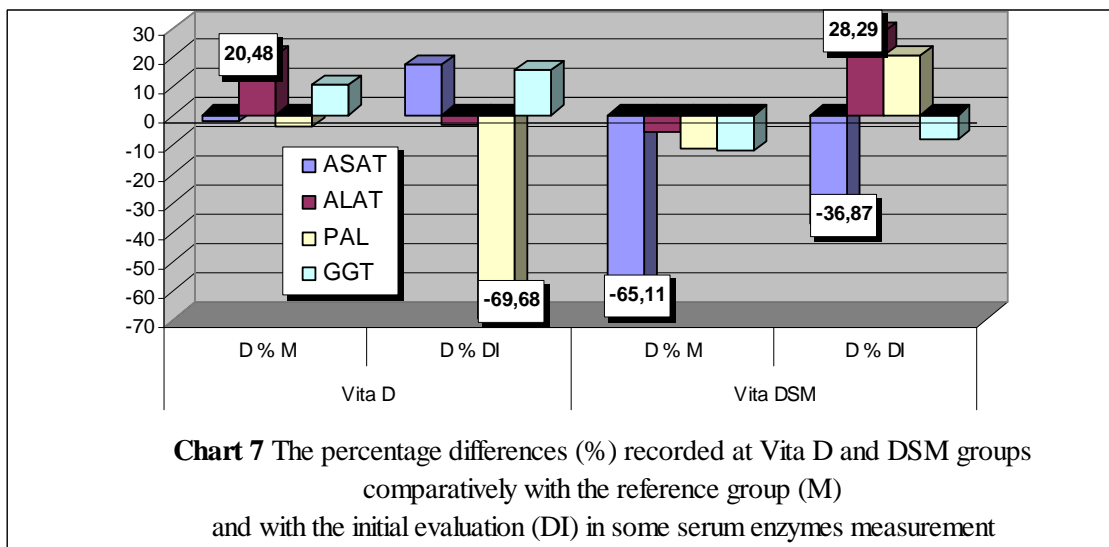


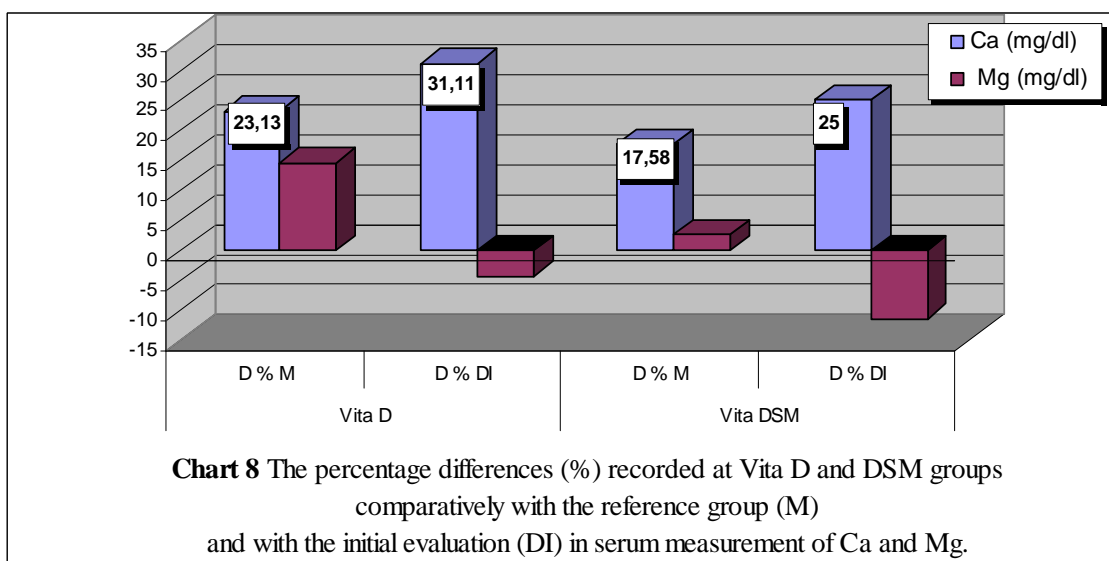
Chart 6 The percentage differences (%) recorded at the Noritake D and DSM groups comparatively with the reference group (M) and with the initial evaluation (DI) in serum proteins measurement.

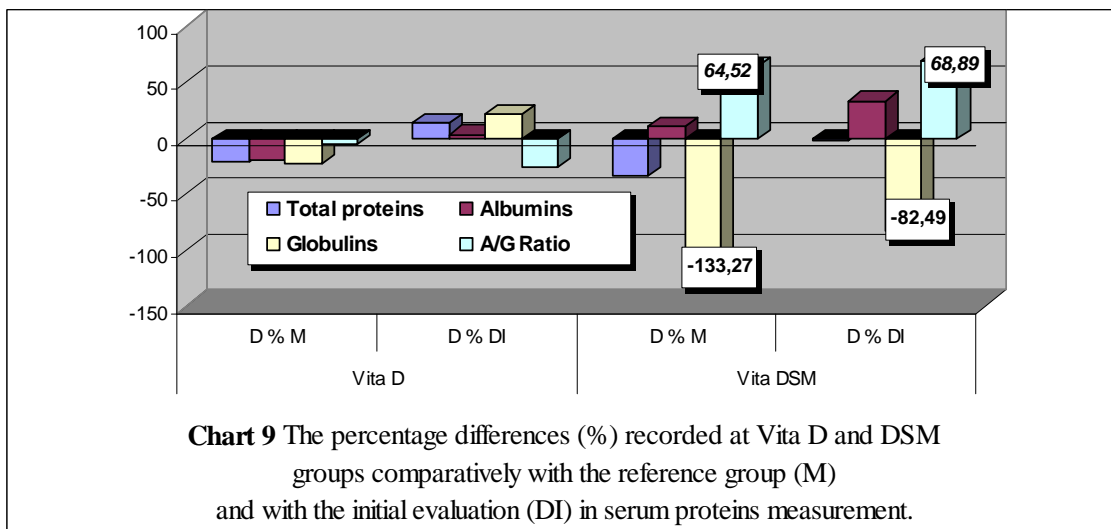


The Vita D and Vita DSM groups didn't count any significant modifications regarding the biochemical parameters explored, nor compared to control group neither to literature values.

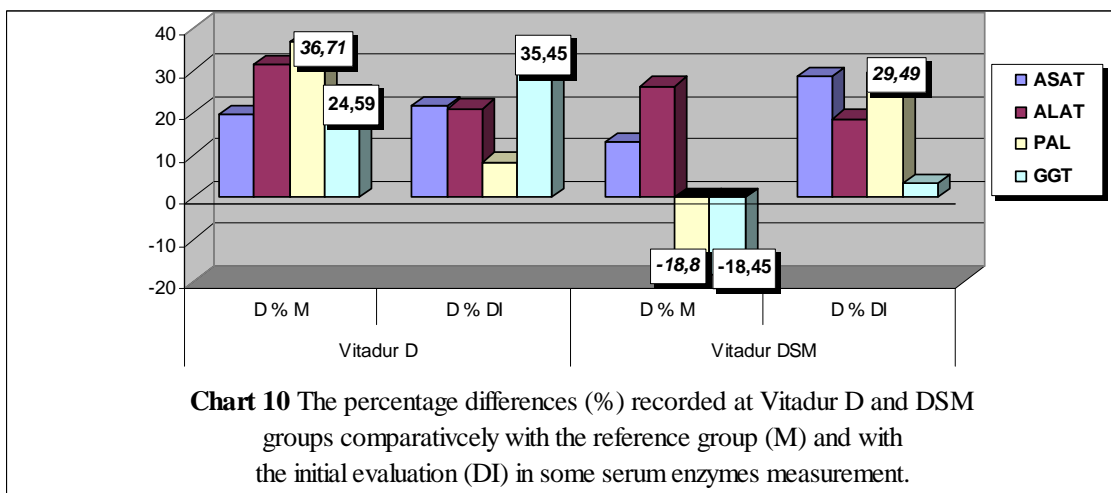
Although, we encounter some large variations in serum enzymes (Chart 7), whose differences oscillate between -69,68% in Vita D compared to DI (PAL) and -65,11% in Vita DSM compared with control (ASAT). In the case of minerals investigated (Chart 8), the calcium level increase constantly, both in Vita D and in Vita DSM groups, compared with both control group (+23,13% and +17,58%) and with the initial evaluation (+31,11% and +25%). The serum proteins values (Chart 9) are very close to those obtained in the control group, even if one can observe a severe decrease of serum globulins level in Vita DSM group, comparatively with the reference group (-133,27%) and with the initial evaluation (-82,49%).

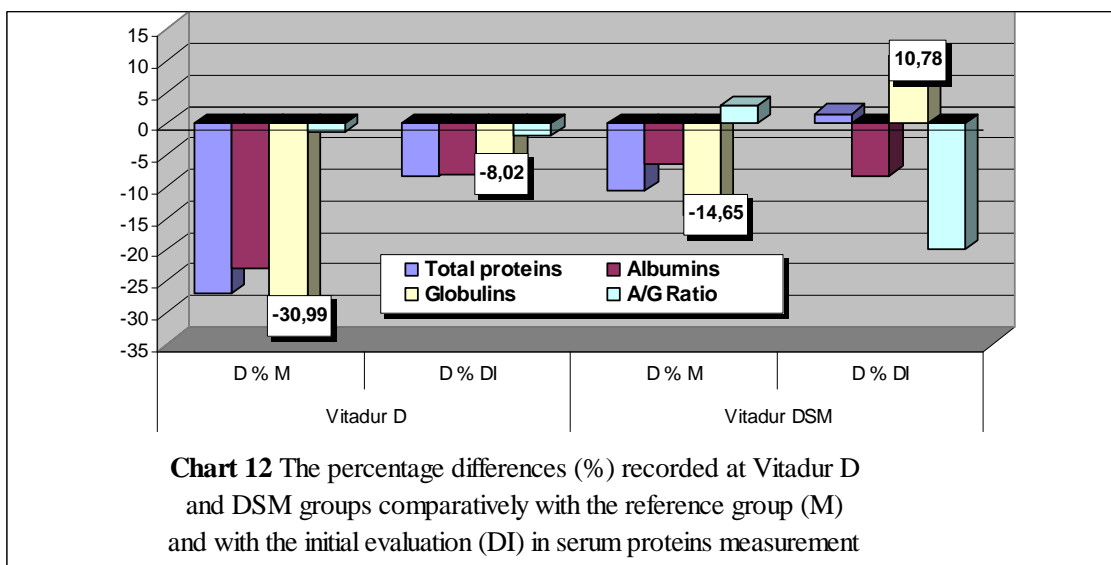
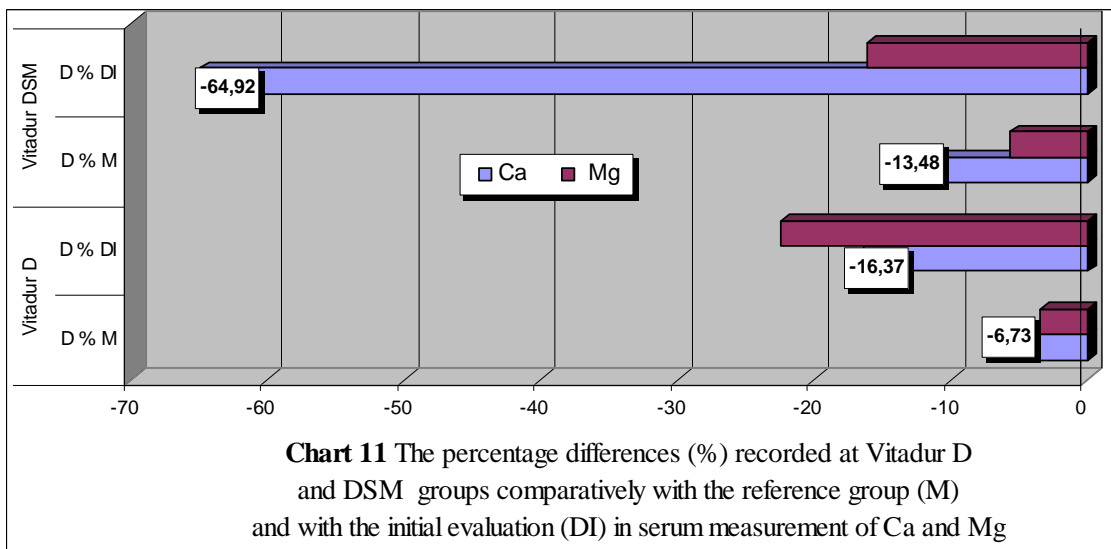
This fact determines a significant increase of A/G ratio in this experimental group (+64,52% compared to control group and +68,69% compared to the initial evaluation).





The situation remains the same in the Vitadur D and Vitadur DSM groups, in the sense that, there are still some positive differences in the serum enzymes concentration (Chart 10) compared with control group (+36,7% PAL and +24,59% GGT in Vitadur D) and compared to initial evaluation (ex: +34,45 GGT in Vitadur D). The determination of the serum minerals concentration evidences a constant decrease of Ca²⁺ and Mg²⁺ values, especially in Vitadur DSM, compared with reference group (-13,48%) and with the initial evaluation (-64,92%).





But, despite these relative large differences recorded, the enzymes, minerals and proteins serum values falls within the normal oscillation, according to data from literature.

CONCLUSIONS

This study was aimed to test the biocompatibility of some dental composites (Ivoclar, Noritake, Vita, Vitadur) in contact with animal tissues, from the point of view of the changes that affect the level of certain serum biochemical parameters (enzymes, minerals, proteins).

Taking into consideration the aspects discussed above, we can conclude that there were not recorded pathological changes of the biochemical parameters that have been explored.

We can say, without any doubt, that the human dental materials we studied are safe to be use, both from the point of view of clinical and of the serum biochemical reactions.

REFERENCES

1. Domingo JL, Gomez M, Bosque MA, Corbella J, 1989, Lack of teratogenicity of aluminum hydroxide in mice. *Life Sci*; 45: 243-247
2. Johnson-Delaney Cathy, 1995, *Exotic Companion Medicine: Handbook for Veterinarians*, Wingers publishing, INC, 12-29.
3. Marcus I, 2004, *Biologia și Patologia Animalelor de Laborator*, Editura Roprint, Cluj.
4. Moore JE, McCulloch WT, 1974, The inclusion of radioactive compounds in dental porcelains. *Br Dent* 1974; J 36:101-106
5. Olsson KG., Furrts B., Anderson B., Carlsson GE, 2003, A long term retrospective and clinical follow-up study of In Ceram Alumina FPDs *Int J Prosthodont*;16:150-156
6. Quireneb M, van Steenberghe D, 1993, Bacterial colonization of the internal part of two stage implants. An *in vivo* study. *Clin Oral Implants Res* 4,158-161.
7. Ruano R, Jager RG, Jager MMMm 2000, Effect of a ceramic and nonceramic hydroxiapatite on cell growth and procollagen synthesis of cultured human gingival fibroblasts. *J Periodontol*; 71: 540-545.
8. Steffik DE, Corpe RS, Buttel K, 1998, *In vivo* evaluation of the biocompatibility of implanted biomaterials: morphology of the implant-tissue interaction. *Implant Dent*; 7: 338-350.
9. Tateishi T, Yunoki H. Research and development of alumina and zirconia artificial hip joint. *Clin Mat* 1993;012:219-225.
10. Tinschert J, Zwez D, Marx R, Anusavice KJ, 2000, Structural reliability of alumina-feldspar, leucite, mica and zirconia based ceramica. *J Dent*;28: 529-535
11. Ukamoto Y, Horibe T, 1984, Physical properties and color analysis of dental porcelain. *Dent Mater J* 3:148-162
12. Veronese I, Guzzi G, Giussani A, Cantone C, Ripamonti D, 2006, Determination of doze rates from natural radionuclides in dental materials. *J Environ Radioact*; 91: 15-26
13. Zelic O, Dimitrijevic B, Vasilijevska M, Dujic A, Lecik PC, 1998, A dental implant: aluminium trioxide exhibited no effect on mouse reproductive and mutagenic potential. *J Clin Periodontol*; 25: 892-896
14. Wunderlich RC, Yaman P, 1986, The *in vitro* effect of topical fluoride on dental porcelain. *J Prosthet Dent* 55:385-388.
15. Wang ML, Tulli R, Manner PA, Sharkey PF, Hall DJ, Tuan RF, 2003, Direct and indirect induction of apoptosis in human mesenchymal stem cells in response to titanium particles. *J Ortop Res*;21:697-707