Comparison of the Histopathological Effects of Subcutaneously Implanted Metallic and Silicon with Metallic Slot Brackets in Rats

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Abstract: The majority of orthodontic alloys contain nickel. The release of nickel can cause allergy and may lead to hypersensitivity. ***Aim*** The study was conducted to compare the histochemical effects of subcutaneously implanted metallic and silicon with metallic slot brackets in rats. ***Material and methods:*** The study was conducted using 30 rats. They were equally divided into three groups (10 each). The first group was the control group. In the second group, metallic bracket was subcutaneously implanted. In the third one, silicon bracket with metallic slot was similarly implanted. Before scarifying the animals, blood sample from the renal artery was taken. All animals were sacrificed after 15 days after implantation. Tissue samples were taken from the area around the bracket and from the spleen. They were microscopically examined. ***Results:*** The differential leucocytic count revealed significant ($P \leq 0.05$) increase of monocytes and lymphocytes in both in the second and third groups. However, the increase was more in the second group. Concerning basophils count the significant increase was seen among the second group only. Concerning microscopic examination, the implantation site in the second group showed granulation tissue prominently infiltrated with lymphocytes and macrophages. Abundant edema separating fibroblasts, macrophages and lymphoid cells with scanty connective tissue fibers were also observed. Lymphocytes in follicular and parafollicular area in the spleen were seen. Viewing the third group, A few amounts of lymphocytes and monocytes as well as mature fibrous connective tissue were observed at the site of implantation. The white pulp in most cases appeared more or less normal. Only, mild proliferation of lymphocytes in the perifollicular area was observed. **Conclusions:** Both brackets exhibited inflammatory response. However, inflammation was more severe in the second group. This was attributed to higher metallic content (nickel) which can induce more severe immune reactions.

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Key words: Nickel, brackets, immunology, spleen, microscopic analysis

1. Introduction

Despite the great progress in orthodontic treatment, undesirable effects could not be prevented. All orthodontic alloys except for β-Ti alloy and pure Titanium contain nickel. The release of the latter produces mild allergic reactions. This has to be of clinical concern.

The discharge of nickel ions, which is a strong immunologic sensitizer, may result in contact hypersensitivity[1]. Adverse responses have been reported occasionally such as stomatitis, gum hyperplasia, cheilitis, labial desquamation, and erythemamultiforme[2]. Metal-sensitive patients can present symptoms ranging from contact allergy to autoimmune disease[3].

Beside contact dermatitis, higher risks of discomfort in the mouth, making treatment and hygiene difficult were also recorded[4]. The fact of nickel release in saliva and blood suggests rising up of allergic responses[5] . Messer *et al.,* [6] in an *In- vitro* experiment on cultured human gingival fibroblasts, showed that ions released from implanted nickel-chromium alloys can cause altered cellular functions.

In an explanation of the effect of released nickel, Hensten-Pettersen *et al.,* [7] claimed that the absorbed nickel binds to certain proteins and forms antigens that, in turn, when in contact with the T lymphocytes of the regional lymph nodes, result in the formation of activated specialized T-cells. These T-cells are capable of causing tissue damage once brought into the blood circulation by lymph vessels.

Moreover, Setcos *et al.,*[8] advocated that the nickel allergy comprises Type IV hypersensitivity reactions which are cell-mediated by T lymphocytes. These cells are an important component of the immune response to many intracellular pathogens and some non degradable antigens.

The effect of nickel release is still debatable. Many authors claimed that it has cytotoxic effects [9]. Other authors indicated that Nickel concentration in serum and saliva from patients who wear fixed orthodontic appliances is similar to those found in healthy individuals[5].

The continuous exposure to nickel alloys might lead to oral tolerance mechanisms that modulate nickel sensitivity, as evidenced by the lower cell proliferation index in patients undergoing orthodontic treatment over 24 months. However, further studies are needed to clarify the major cell phenotype associated with the immune response[4].

The release of nickel is related to the surface area, exposure time, and environment; but there seems to be no exact knowledge of the type and duration of oral exposure needed to elicit the induction of tolerance[10]. As known, hypersensitivity results from the action of the immune system against an antigen leading to sensitivity. Nickel plays the greatest part of importance in this issue not only in Orthodontics but also in other fields even with ear piercing[11, 12].

As a result, the innovation of new types of brackets (nickel free) so as to decrease any unexpected or undesirable effect of nickel was mandatory. Esthetic brackets: acrylic, silicone and ceramic were introduced in the market. However, the problem of friction constituted a major problem in the sliding mechanics and metallic slot was suggested. Again, the debate about hypersensitivity rose up.

The biocompatibility of dental materials is, nowadays, of great concern. There is special interest to the reactions secondary to the use of certain metals in orthodontics. Austenitic stainless steel contains 18% chromium and 8% nickel [13, 14]. These metals are known worldwide as the most sensitizing agents[15]. However, not only metals but also natural rubber latex of disposable hand gloves proved to be potentially allergenic material[16].

Leite and Bell[17] claimed that tissue reactions to the materials most often used in orthodontic treatment may involve either localized irritation of skin and oral mucosa, localized or generalized manifestations of hypersensitivity. Moreover, Synodinos *et al.,*[18] concluded that manifestation of hypersensitivity reactions to materials usually used during orthodontic treatment, may develop into a serious health hazard for allergic or predisposed to allergic reactions patients. So, this study was carried out to assess the histopathological effects of subcutaneously implanted metallic and silicon with metallic slot brackets in rats.

2.Material and Methods:

Fifty Sprague Dawley male rats were brought after birth. They were kept during the lactation period in the same environment. After 7 days from the feeding period (15 days), thirty rats were selected. The weight of the selected rats was 177-224 g. For standardization, each rat was kept in a separate cage. Cages had the same dimensions. Their floor was covered by wood chips. All rats were fed standard rat chow and water *ad Librium*.

The thirty rats were equally divided into three groups (10 each). The first group constituted the control group. Metallic brackets (American Orthodontics, Sheboygan, Wisconsin, USA) were subcutaneously implanted in rats of the second group. The chemical composition of the bracket is illustrated in Table 1. In the third group, silicon bracket with metallic slot (Ormco, Glendora, California, USA) was subcutaneously implanted. In both groups, upper central 0.022 inch brackets were used.

Table 1: A table provided from American Orthodontics (AO) showing the chemical composition of the bracket used in this study.

Element	C	Mn	Si	P	S	Cr	Ni	M	Cu	Fe
Percentage %	0.05	1	1	0.03	0.03	14/16	5/7	0.5/1	1.25/1.75	balance

Implantation procedure:

At start, brackets were disinfected by immersion in lysoformine 10% for 10 min then rinsed with distilled warm water and vacuously dried for 30 seconds.

Animals of the second and third groups were left in closed small plastic rooms of dimensions 20 x10 x 8 cm. All animals received intraperitoneal anesthesia (Ketamine, 50 mg/kg b.wt and Xylazine, 10 mg/kg b.wt).

A 3 x 3 cm surface was shaved on the animal's back. (Fig 1) The superficial area was then disinfected using betadine solution. An incision of 1 cm was made longitudinally . (Fig 2) The incision was performed using bard barker blade. The area was then widened to expose the muscle above which the disinfected bracket was placed. (Fig 3) The incision was sutured (Fig 4) and the animal received an analgesic injection (Sodic dipyrone, 0.3 ml/100 g weight). The animal was held to its cage. All animals were sacrificed after 15 days.

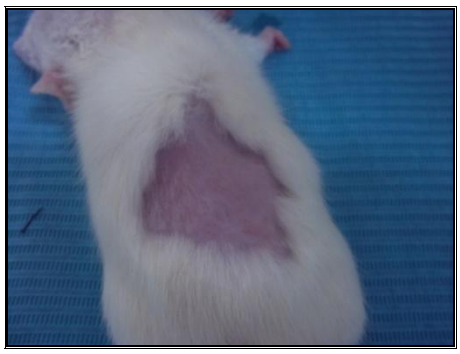

Fig 1: The animal's back shaved

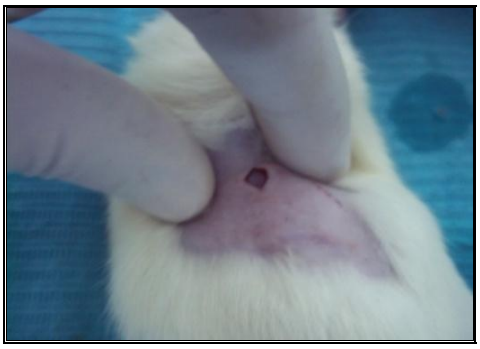

Fig 2: 1 cm incision on the back

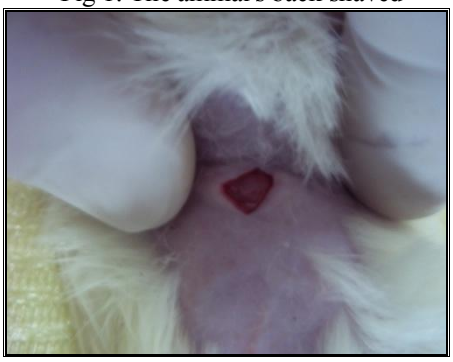

Fig 3: Widening to expose muscle

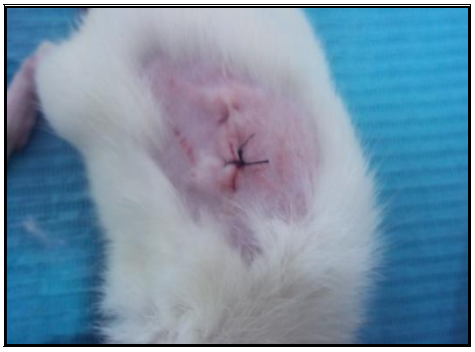

Fig 4: Sutured incision

Samples collection:

A-Blood Samples:

Before sacrificing the animals, blood was aspirated from the retinal artery. Blood was then collected in a test tube containing EDTA anticoagulant. Five ml were collected. Five drops were put on five slides and fixed using methyl alcohol 100% for 3 min. The sample was then rinsed with distilled water and placed in a holder containing May-Grünwald Giemsa stain for 20 min. After staining, the sample was again rinsed with water then dried.

B-Tissue samples:

The tissue samples were collected from the skin and subcutaneous area that surrounded the implanted bracket and from the spleen. The collected samples were preserved in pure formaline. Using microtome, the sample was cut into small sections; each nearly 5 micrometer. The samples were stained using Hematoxylin and eosin (H & E) and were viewed under 25 x magnification.

3.Results:

A-Differential blood analysis:

Data were checked, entered, and analyzed using the Statistical Package for Social Sciences (SPSS), version 1.7 software (IBM, Armonk, NY, USA, ®SPSS Inc, Chicago, IL, USA). Mean, standard deviation, standard error as well as the 95% confidence interval were calculated. The 95% confidence interval determines the expected values between which 95% of the data lie. All these values (descriptive statistics) are illustrated in Table 2. One way analysis of variance (ANOVA) test was depended on to detect any significant difference between the groups. The mean difference was considered significant at the level 0.05. (Table 3)

Viewing the differential leucocytic count, a significant increase ($P \leq 0.05$) in neutrophils and lymphocytes in the second group was detected. This was also the case with the third group. Comparison between the second and third groups revealed that the count was higher in the second one. No significant difference was detected among monocytes and eosinophils. Concerning basophils, the only change was detected among the second group where there was a significant increase. No significant difference was detected between the third and the control group. The comparison of blood analysis among the three groups is illustrated in Fig 5.

B- Histopathologic analysis:

Metallic brackets group:

Implantation site:

Examination of the site of implantation after 15 days revealed prominent mononuclear to severe mononuclear cell infiltration with presence of abundant amount of fibroblast cell. However, the collagen fibers are scanty. Numerous blood vessels

as well as prominent edema isolating the elements were seen in the area. Necrotic tissue was sometimes observed in the area of implantation. Large blood vessels surrounded by macrophages were frequently seen in most cases. Scanty fibrosis was a prominent feature of this period (Figs. 6, 7).

Table 2. Descriptive statistics

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Lower Bound		
Neutrophils	**Gp I**	10	62.120	1.5271	.6829	60.224	64.016	60.4	64.2
	Gp II	10	69.320	2.3732	1.0613	66.373	72.267	66.0	71.8
	GpIII	10	63.560	1.7053	.7626	61.443	65.677	61.2	65.4
	Total	30	65.000	3.6707	.9478	62.967	67.033	60.4	71.8
Lymphocytes	**Gp I**	10	32.72	0.832	0.372	31.69	33.75	32	34
	Gp II	10	34.92	0.971	0.434	33.71	36.13	34	36
	GpIII	10	32.16	1.493	.668	30.31	34.01	30	34
	Total	30	33.27	1.620	.418	32.37	34.16	30	36
Monocytes	**Gp I**	10	3.360	.4561	.2040	2.794	3.926	2.6	3.8
	Gp II	10	3.960	1.0431	.4665	2.665	5.255	2.6	5.0
	GpIII	10	3.360	0.9737	0.4354	2.151	4.569	2.4	5.0
	Total	30	3.560	0.8526	0.2201	3.088	4.032	2.4	5.0
Eosinophils	**Gp I**	10	0.720	0.1789	0.0800	0.498	0.942	0.6	1.0
	Gp II	10	0.680	0.1095	0.0490	0.544	0.816	0.6	0.8
	GpIII	10	0.560	0.3286	0.1470	0.152	0.968	0.2	1.0
	Total	30	0.653	0.2200	0.0568	0.532	0.775	0.2	1.0
Basophils	**Gp I**	10	0.520	0.2280	0.1020	0.237	0.803	0.2	.8
	Gp II	10	0.840	0.3286	0.1470	0.432	1.248	0.6	1.4
	GpIII	10	0.360	0.1673	0.0748	0.152	0.568	0.2	.6
	Total	30	0.573	0.3105	0.0802	0.401	0.745	0.2	1.4

Table 3. ANOVA test indicating the significance between groups

Dependent variables	(I) material used	(J) material used	Mean difference	Std. Error	Sig	95% Confidence Interval for Mean	
						Lower Bound	Lower Bound
Neutrophils	**Gp I**	**Gp II**	-7.2000*	1.2040	**.000**	-10.546	-3.854
		Gp III	-1.4400	1.2040	.764	-4.786	1.906
	Gp II	**Gp I**	7.2000*	1.2040	**.000**	3.854	10.546
		Gp III	5.7600*	1.2040	**.001**	2.414	9.106
	Gp III	**Gp I**	1.4400	1.2040	.764	-1.906	4.786
		Gp II	-5.7600*	1.2040	**.001**	-9.106	-2.414
Lymphocytes	**Gp I**	**Gp II**	-2.200*	.718	**.029**	-4.19	-.21
		Gp III	.560	.718	1.000	-1.43	2.55
	Gp II	**Gp I**	2.200*	.718	**.029**	.21	4.19
		Gp III	2.760*	.718	**.007**	.77	4.75
	Gp III	**Gp I**	-.560	.718	1.000	-2.55	1.43
		Gp II	-2.760*	.718	**.007**	-4.75	-.77
Monocytes	**Gp I**	**Gp II**	-.6000	.5470	.883	-2.120	.920
		Gp III	.0000	.5470	1.000	-1.520	1.520
	Gp II	**Gp I**	.6000	.5470	.883	-.920	2.120
		Gp III	.6000	.5470	.883	-.920	2.120
	Gp III	**Gp I**	.0000	.5470	1.000	-1.520	1.520
		Gp II	-.6000	.5470	.883	-2.120	.920
Eosinophils	**Gp I**	**Gp II**	.0400	.1424	1.000	-.356	.436
		Gp III	.1600	.1424	.849	-.236	.556
	Gp II	**Gp I**	-.0400	.1424	1.000	-.436	.356
		Gp III	.1200	.1424	1.000	-.276	.516
	Gp III	**Gp I**	-.1600	.1424	.849	-.556	.236
		Gp II	-.1200	.1424	1.000	-.516	.276
Basophils	**Gp I**	**Gp II**	-.3200	.1583	.198	-.760	.120
		Gp III	.1600	.1583	.996	-.280	.600
	Gp II	**Gp I**	.3200	.1583	.198	-.120	.760
		Gp III	.4800*	.1583	**.031**	.040	.920
	Gp III	**Gp I**	-.1600	.1583	.996	-.600	.280
		Gp II	-.4800*	.1583	**.031**	-.920	-.040

*The mean difference is significant at the 0.05 level.

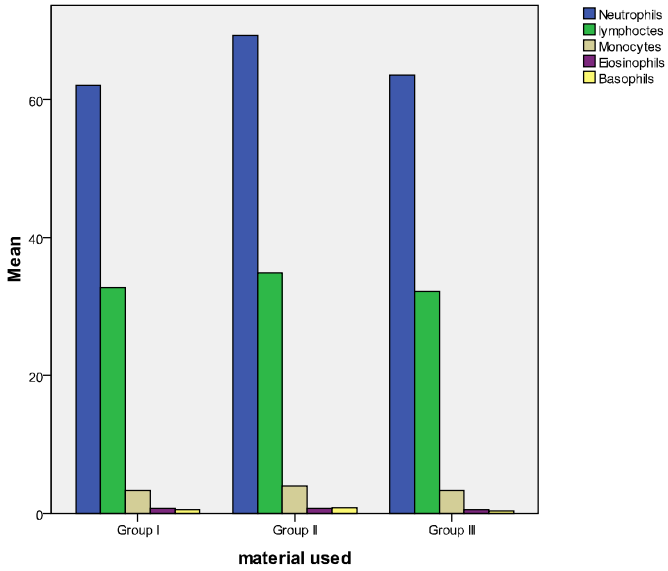

Fig. 5. Comparison of the differential blood count among the three groups

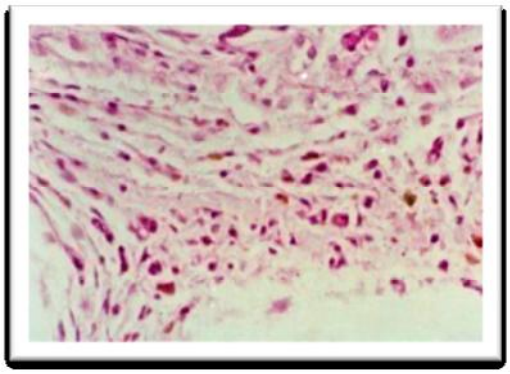

Fig. 6. Granulation tissue prominently infiltrated with lymphocytes and macrophages

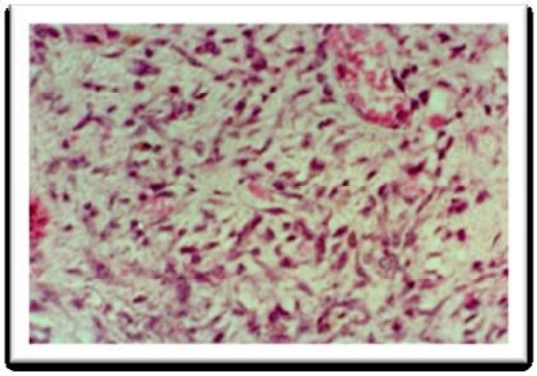

Fig. 7. abundant edema separating fibroblasts, macrophages and lymphoid cells with scanty connective tissue fiber connective tissue fibers

Spleen:

Both the white pulp and the red pulp of the spleen were extensively populated with lymphoid cells. Numerous mitotic activity were observed in the white pulp indicating active germination of splenic follicle in both follicular and parafollicular area (Fig. 8).

Silicon bracket with metallic slot group:

Site of implantation:

The site of implantation appeared as agranulomatous tissue reaction in which fibroblasts were the main constituent elements. However, few mononuclear cells (lymphocytes and monocytes) were observed. Numerous small well developed blood vessels were also seen in the vicinity of the area of granulation tissue reaction (Fig 9) .

In other cases, the site of reaction appeared as a diffuse area of fibrosis in which well developed blood vessels were observed. The main reacting cells were fibrocytes and collagen fibers. Few or no mononuclear cells were sometimes observed (Fig. 10) .

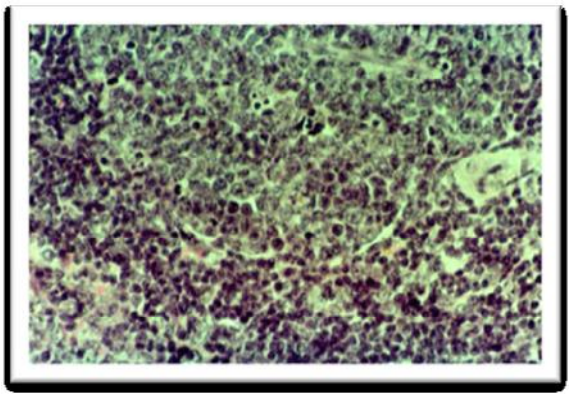

Fig. 8. Lymphocytes in follicular and parafollicular area

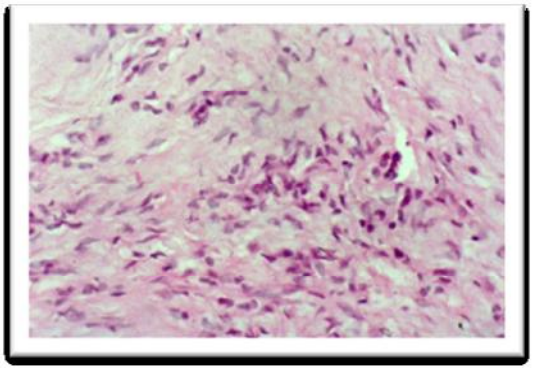

Fig. 9. Connective tissue reaction with few monocytes

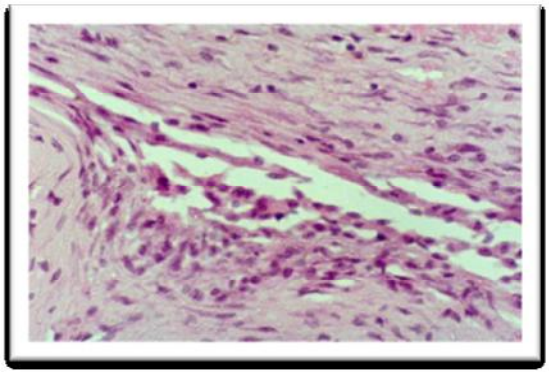

Fig. 10. Diffuse reaction with mature fibrous amounts of lymphocytes and connective tissue

In some cases, the amount of mononuclear cells (lymphocytes) was relatively increased on the expense of fibroblast and collagen fibers. In such cases, the proliferating connective tissue appeared moderately cellular and the mononuclrear cells were diffusely infiltrating it. Well developed blood vessels sometimes containing RBCs were also seen in the proliferating fibrous tissue.

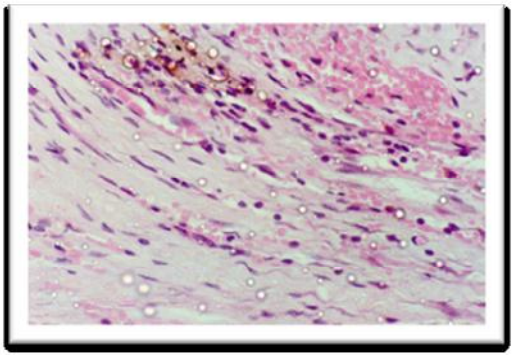

Fig. 11. Increased lymphocytes

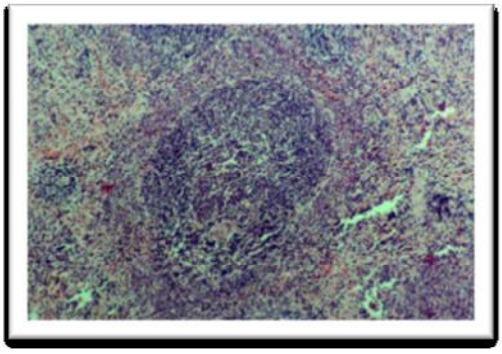

Figure 12

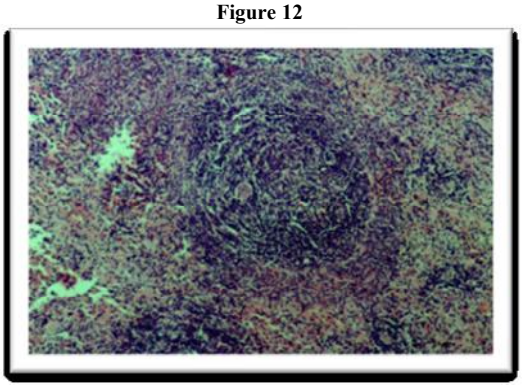

Figure 13

Fig. 12 & 13 showing white pulp of spleen from different rats with more or less lymphocytic proliferation

Spleen:

In the spleen, the white pulp in most cases appeared more or less normal. Only, mild proliferation of lymphocytes in the perifollicular was observed (Figs. 12, 13) .

4. Discussion:

The current study did not stress on hypersensitivity against nickel or other materials or metals included in any alloy used in Orthodontics. This issue was clarified by several studies. Among these are that carried out by Menezes *et al.,* [19] who studied hypersensitivity to metals in orthodontic using a patch test. Moreover, the sex difference was overlooked. The reason is that sexual dimorphism was also put into consideration in various researches. Tsalen and Zaprianov [20] concluded that sensitivity to nickel is 3 to 5 times more common in women than in men. However, Prystowsky *et al.,* [21] Jones *et al.,* [22] and Kerusuo *et al.,* [10] showed that this difference to be up to 10 times greater among female subjects. This was attributed to the use of earrings [10, 23, 24, 25] and other jewelry.[21, 26]

This study stressed on the local immunity to two different types of brackets. Local immunity was determined by studying histopathological changes and tissue reaction at the site of implanted material and an immune organ: spleen. In addition, blood samples were taken. Differential leucocytic count was carried out and compared to other groups.

For standardization, the animals on which the experiments were carried out lived in the same

environment from birth. They took the same food. They had the same weight range. Although no association between allergic reaction to nickel and age was observed [13, 26], they all had the same age.

The special diagnostic tests for allergic predisposition: the ELISA (enzyme-linked immunosorbent assay) and RAST (radioallergosorbent test) tests were not depended upon. The reason is that these tests were proved to have 50-60% Sensitivity. Hammer and Paulson [27] suggested that the result of the test is negative in a significant number of allergic individuals. There may be also a risk of sensitization when applying the patch test [28].

The immune system has evolved to protect us from pathogens. It is the body defense or resistance mechanism against foreign substances and infection entering the body. The ultimate goal of the immunological reaction is to recognize, neutralize, and destroy the foreign agent. The immune response is that response which occurs when foreign substances (antigens) enter the body [29].

The bracket is considered as foreign substance and when grafted subcutaneous it initiated the immune response. The reaction is initiated when the Langerhans cells and mucosal macrophages recognize and present the antigen to T cells leading to T cell activation. The latter cells release lymphokines into the surrounding tissues.

These compounds (IL-2, IFNγ) activated macrophages, which in turn, secrete other cytokines including IL-1, which promotes activation and attraction additional inflammatory cells from the circulation. These cells released the inflammatory mediators which explained the appearance of nflammation (redness and swelling) occurred over the bracket [30, 31].

Viewing the previous statement, the results of the present study can be easily interpreted. The inflammatory response to the foreign body (bracket) is manifested by the increased count of both neutrophils and lymphocytes observed in the second and third groups. The significant difference between the two groups can be attributed to increased metal contact in the bracket of the second group. This is also manifested by the increase count of the basophils in the second group only.

The blood group has to be put into consideration if such a study is going to be conducted on human subjects. The fact of association between blood group and susceptibility to allergic reactions was proved by Vaish *et al.,*[32].

Conversely, Janson *et al.,*[28] results showed no association either with blood groups or Rh factor.

Concerning the histopathological findings, the inflammatory cells (macrophages and lymphocytes) were infiltrating the connective tissue in the second group. In the spleen, lymphoid cells were extensively populating both the white and red pulp. These findings are in accordance with the study conducted by D'Attillio *et al.,*[33] which showed an inflammatory infiltrate, with an overall impression that vasodilatation and leukocyte infiltration predominantly macrophages and plasma cells. The third group did not show such extensive inflammatory response. Again this may be attributed to the low metallic content. However, it has to be put in mind that silicon brackets also induce inflammatory response.

Conclusions:

1- Both the brackets: stainless steel and silicon with metal slot exhibited inflammatory response.
2- The inflammatory response was more exhibited with metallic brackets.
3- The differential leucocytic count (neutrophils and lymphocytes) increased among the second and third compared to the control group.
4- The white and red pulp of the spleen assured the more severe inflammatory response in the second group when compared with the third one.

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