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Research Report

Contact hypersensitivity after tongue piercing

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ABSTRACT

Background: *Recently tongue piercing has become increasingly popular in the society. Several case reports have presented various complications of tongue piercing. However, there is no scientific evidence about contact hypersensitivity to tongue piercing.* ***Purpose:*** *The aim of this study was to investigate the contact hypersensitivity after using tongue piercing.* ***Methods:*** *Nineteen male Rattus norvegicus were divided into three groups: group A treated with vaseline on the back and dorsum tongue (control group), group B (I) treated with $HgCl_2$ 10% cream on the tongue dorsum, group B (II) treated with tongue piercing for 10 days and group C with $HgCl_2$ 10% cream on the back, ear lobe, and tongue, then re-exposure with same materials on ear, back and tongue for 24 and 48 hours. Before and after 24 and 48 hours applications, ear width was measured with sliding caliper. At the end of treatments, the rats were sacrificed. All tissue specimens were made for Hematoylin Eosine (H&E) staining examination. The number of mononuclear cells was counted under light microscope Data was analyzed with One-Way ANOVA followed by LSD ($p<0.05$).* ***Results:*** *The result of this study showed that there were a significant difference of the thickness of ear lobe and the number of mononuclear cells (lymphocyte and monocyte) among all groups.* ***Conclusion:*** *It is concluded that tongue piercing induce contact hypersensitivity.*

Key words: *Contact hypersensitivity, tongue piercing, mononuclear cell*

ABSTRAK

Latar belakang: *Saat ini pemakaian tongue piercing sangat popular di masyarakat.* ***Beberapa laporan kasus menunjukkan bahwa*** *tongue piercing menimbulkan beberapa komplikasi. Namun, belum ada bukti ilmiah mengenai reaksi hipesensitivitas tongue piercing.* ***Tujuan:*** *Untuk mengetahui reaksi hipersensitivitas setelah menggunakan tongue piercing.* ***Metode:*** *Sembilan belas tikus jantan Rattus novergicus yang dibagi dalam tiga kemlompok yaitu: grup A diberi perlakuan dengan vaselin pada punggung dan dorsum lidah, grup B (I) diberi perlakuan dengan krim $HgCl_2$ 10% pada dorsum lidah dan B (II) perlakuan tongue piercing selama 10 hari. Grup C diberi perlakuan dengan $HgCl_2$ pada punggung, daun telinga, dan dorsum lidah, kemudian diberi perlakuan ulang dengan bahan dan tempat yang sama selama 24 dan 48 jam. Sebelum dan setelah perlakuan selama 24 dan 48 jam ketebalan telinga diukur dengan sliding caliper. Setalah perlakuan tikus didekapitasi kemudian dibuat preparat jaringan untuk pemriksaan hematoxilin & eosin (H & E). Perhitungan jumlah sel mononuclear dilakukan menggunakan mikroskop cahaya.* ***Hasil:*** *Penelitian ini menunjukkan bahwa terdapat perbedaan ketebalan telinga dan jumlah sel mononuklear yang bermakna setelah perlakuan antar kelompok pada hasil analisa dengan menggunakan ANOVA dan LSD ($p<0.05$).* ***Kesimpulan:*** *Tongue piercing dapat menginduksi reaksi hipersensitivitas kontak.*

Kata kunci: *Hypersensitivitas kontak, tongue piercing, mononuclear cell*

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INTRODUCTION

Body piercing is an art of the human body that has existed since many centuries ago and became a symbol of pride of tribes.[1] In recent years, body piercing tends to increase in most communities.[2] Tongue piercing is one of the oral piercing which is the most increasingly used by teenagers to express his or her identity.[3-5] Oral piercing is the insertion of metal which has a barbell with varying in size 12-18 mm intraoral and perioral.[6] In general, the metal in tongue piercing is made from stainless steel[7] and can be also derived from surgical stainless steel,[6] silver, gold-plated surgical stainlees steel, and plastic.[8]

Various case reports related to the use of tongue piercing has shown the existence of various complications. Allergic reactions have been reported in some cases of oral piercing, especially in nickel-contained metal.[9] Silver-contained metal can release abrasive material that may cause infection and allergic reactions,[8] The most complication of tongue piercing was contact dermatitis.[4,10,11] However there have never been studies that prove the contact hypersensitivity to the metal of tongue piercing.

Hypersensitivity reaction to the tongue piercing may associate with the metallic contained of material in a tongue piercing. Metal contained-tongue piercing that may induce allergy is nickel, or alloy containing nickel and cobalt. Chromates have also been reported as a metal that causes allergies.[7] Contact hypersensitivity has been also known as type of slow hypersensitivity, cell mediated immunity (CMI), delayed type hypersensitivity (DTH), cell mediated immunity (CMI), delayed type hypersensitivity (DTH) or a reaction to the tuberculin which is established more than 24 hours after the body exposed to allergens. Contact hypersensitivity is a response of T cells that have been desensitized to the particular antigen. This resulted in sensitized T cells that will release lymphokine which acts as a mediator of delayed type hypersensitivity. The manifestations of this reaction are infiltration of monocytes and lymphocytes, or macrophages and cause tissue swelling at the site of antigen.[12] In animal models, manifestation of hypersensitivity can be seen from the swelling in the ear lobe.[13]

Wistar rat *(Rattus norvegicus)* is one of animal model which widely used for research in Dentistry and Medical science. Many studies have used Wistar rats *(Rattus norvegicus)* as animal model such as hypersensitivity reactions to mercury (Hg),[13] the influence of cold cured *acrylic* resin monomer[14] and the contact hypersensitivity of *Aloe vera's* gel.[15] Wistar rats have biological system which relatively similar with human body. Another consideration was the Wistar rat is bigger than mice and more easily to handle.[13,15,16] Previous study of tongue piercing had been done in Sprague dawley,[16] and other studies used Beagles dog,[17] but the study of contact hypersensitivity of tongue piercing has never been reported. This aim of this study is to investigate contact hypersensitivity after the use of tongue piercing. This research may benefit for development of science, particularly in oral medicine, information for clinician, tongue piercing users and the community about the potential harmful effect of the use of tongue piercing on immune system of body.

MATERIALS AND METHODS

The method of this study was similar which is described in previous study with some modifications.[13-16] Nineteen male rats Wistar (*Rattus norvegicus*) were divided into three groups: negative control (A), treatment (tongue piercing) (B), and positive control ($HgCl_2$) (C). There are three phases of the treatment of this study, namely phase of sensitization to tongue piercing and $HgCl_2$, phase of re-exposure on the back and right ear, and determination phase hypersensitivity reaction after 24 hours (group B1 & C1) and 48 hours (group B2 & C2) of re-exposure. Group A was treated with vaseline (negative control group). Group B was the treatment group (tongue piercing). Group C was a positive control group with treated with $HgCl_2$. All treatments carried out in Wistar rats for 10 days for sensitization then performed the same treatment on the back and ear lobe after 24 hours (day 11) and 48 hours (day 12). The thickness of the ear was measured before and after re-exposure 24 and 48 hours. Six hours later, all rats were decapitated.

Tissue specimen from ears, back and tongue's Wistar in each group were taken and made a tissue slide for HE staining examination to count mononuclear cells (lymphocytes and monocytes). Mononuclear cells mean were obtained from seven different views using a light microscope with a magnification of 100x. All procedure of this study has been approved by the Committee of Ethics of Medical Research and Health Faculty of Medicine Gadjah Mada University Yogyakarta. The mean difference of ear lobe thickness and the number of cells mononuclear (lymphocytes and monocytes) in all groups were analyzed with One-Way ANOVA and Least Significant Difference (LSD).

RESULTS

Clinically, contact hypersensitivity can be shown in the difference of the ear lobe thickness after treatment (tongue piercing and $HgCl_2$). The ear lobe thickness increased after tongue piercing for 24 hours (Figure 1a) and for 48 hours (Figure 1b). The ear lobe increased after $HgCl_2$ treatment for 24 hours (Figure 1b). The thickness of ear lobe after treatment showed a significant higher than before treatment (Table 1 & 2).

The significant difference of the thickness of ear lobe was shown between tongue piercing treatment for 24 hours (group B1), tongue piercing treatment for 48 hours (group B2), and $HgCl_2$ treatment for 24 hours (group C1). After comparing all groups, the result of LSD analysis (Table

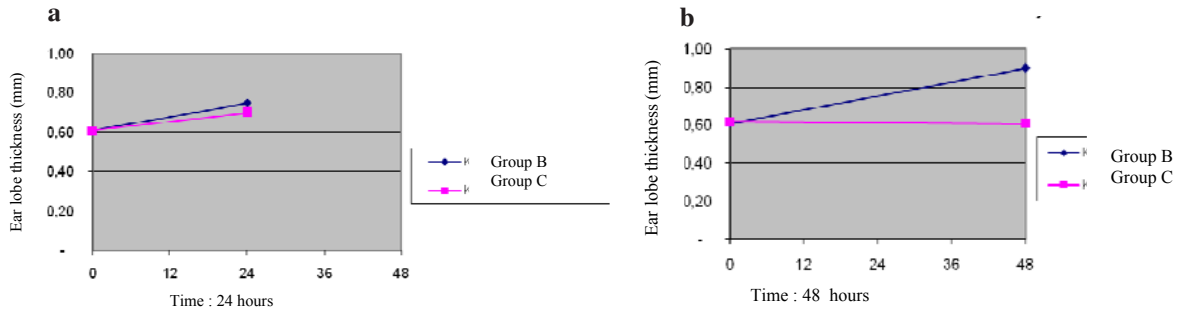

Figure 1. Changes in the thickness of the ear before and after treatment. a) 24 hours in group B and C, b) 48 hours in group B and C.

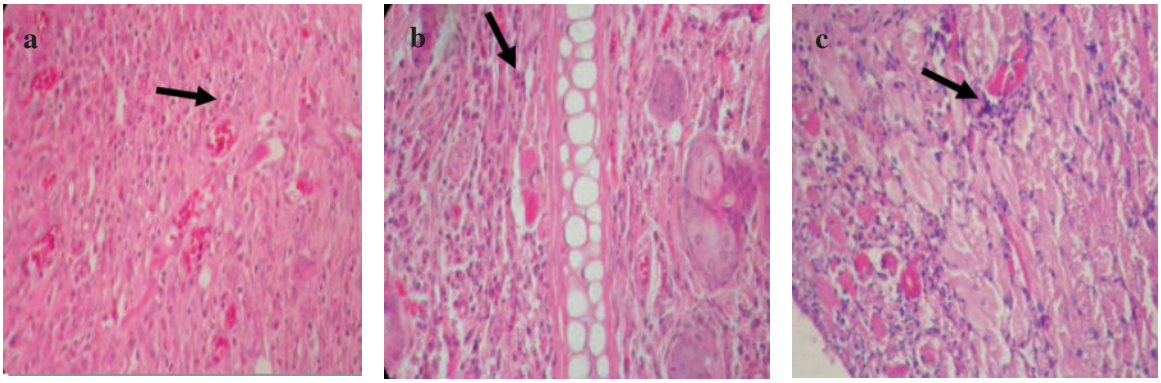

Figure 2. Infiltration of mononuclear cells (black arrows). a) back, b) ear, c) tongue after tongue piercing treatment 24 hours (microscope, magnification 100×, HE).

Table 1. Comparison of ear lobe thickness before ad after treatment in all groups (mm)

Group	Before	After	Mean Difference + SD
	Mean ± SD	Mean ± SD	
Group A	0.63 ± 0.05	0.62 ± 0.02	0.01 + 0.02
Group B1	0.61 ± 0.02	0.75 ± 0.05	0.14 + 0.14*
Group B2	0.61 ± 0.02	0.90 ± 0.14	0.29 + 0.29*
Group C1	0.61 ± 0.07	0.70 ± 0.07	0.09 + 0.10*
Group C2	0.62 ± 0.02	0.61 ± 0.02	0.01 + 0.02

* significantly difference ($p<0.05$)

Table 2. LSD test result between all groups

Group	p
A and B2	*0.01
B1 and B2	*0.02
B1 and C2	*0.05
B2 and C1	*0.01
B2 and C2	*0.01

* Significantly difference ($p<0.05$)

2) showed there were significant difference of ear lobe thickness between control group (group A) and tongue piercing treatment for 48 hours (group B2), between tongue piercing treatment for 24 hours (group B1) and tongue piercing treatment for 48 hours (group B2), between tongue piercing treatment for 24 hours (group B1) and $HgCl_2$ treatment for 48 hours (group C2), between tongue piercing treatment for 48 hours (group B2) and $HgCl_2$ treatment for 24hours (group C1), between tongue piercing treatment for 48 hours and $HgCl_2$ treatment for 48 hours (group C2). Contact hypersensitivity also can be shown by the infiltration of mononuclear cells (arrow) in treatment group (B1 & B2) and positive control group (C1 & C2) (Figure 2).

The infiltration of mononuclear cells (monocytes) was shown in the site of tongue piercing. The picture was an example that the increased number of monocytes (arrow) was shown in the connective tissue as response to the tongue piercing treatment. According to One-Way ANOVA, there were a significant difference in the number of mononuclear cells mean between all treatment groups (B), negative control group (A) and positive control group (C) ($p<0.05$).

Table 3 showed that the means of monocytes between tongue piercing treatment groups (group B) were higher than HgCl2 treatment groups (group C). The result of ANOVA showed that there was a significant difference ($p<0.05$) of the number of monocyte between all treatment groups in every part of treatment site.

Table 4 showed that the means of lymphocytes between tongue piercing treatment groups (group B) were higher than $HgCl_2$ treatment groups (group C). The result of ANOVA showed that there was a significant difference

($p<0.05$) of the number of lymphocytes between all treatment groups in every part of treatment site. The results of Least Significant Difference (LSD) showed there were significant differences between both control groups and treatment group (Table 3, 4 & 5).

Table 3. The mean of monocyte in all groups

Treatment	Back	Ear	Tongue
	Mean ± SD	Mean ± SD	Mean ± SD
Group A	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Group B1	2.39 ± 1.07	2.21 ± 0.99	2.39 ± 1.07
Group B2	2.89 ± 0.69	1.36 ± 1.28	2.21 ± 1.17
Group C1	0.96 ± 0.79	1.07 ± 0.77	1.14 ± 0.80
Group C2	2.00 ± 1.52	1.36 ± 1.10	1.36 ± 1.06
p	0.01	0.01	0.01

Table 4. The mean of lymphocyte in all groups

Treatment	Back	Ear	Tongue
	Mean ± SD	Mean ± SD	Mean ± SD
Group A	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Group B1	2.43 ± 1.10	2.79 ± 1.20	2.68 ± 1.12
Group B2	3.07 ± 0.86	1.68 ± 1.16	3.29 ± 1.41
Group C1	1.15 ± 2.29	2.68 ± 1.09	3.21 ± 0.88
Group C2	1.25 ± 1.40	1.11 ± 1.20	1.39 ± 1.13
p	0.01	0.01	0.01

Table 5. LSD test result

Between groups	p
A and B2	*0.01
B1 and B2	*0.02
B1 and C2	*0.05
B2 and C1	*0.01
B2 and C2	*0.01

* p = significantly difference ($p<0.05$)

After comparing all groups, the result of LSD analysis (Table 5) showed there were significant difference of between control group (group A) and tongue piercing treatment for 24 hours (group B1), between control group (group A) and tongue piercing treatment for 48 hours (group B2), between control group (group A) and $HgCl_2$ treatment for 24 hours (group C1), between control group (group A) and $HgCl_2$ treatment for 48 hours (group C2), between tongue piercing treatment for 24 hours (group B1) and $HgCl_2$ treatment for 48 hours (group C2), between tongue piercing treatment fro 48 hours (group B2) and $HgCl_2$ treatment for 48 hours (group C2).

DISCUSSION

The results of this study showed changes of thickness of the ear lobe before and after treatment of tongue piercing (group B) and $HgCl_2$ 10% (group C) (Table 1). This finding supported previous study that the manifestation of delayed type hypersensitivity reaction in experimental animals can be seen through swelling in the ear lobe.[13] The swelling of ear lobe is the clinical sign of tissue inflammation in hypersensitivity reaction.[18] Re-exposure in the same area will result in vasodilatation of blood vessels locally then will cause the excessive blood flow.[19] Vasodilatation causes coagulation activation, result in the formation of fibrin then accumulate in the site of inflammation. Deposit fibrin cause induration or swelling,[18] and the swelling causes increased thickness of the ear. Sensitization phase can occur 7–10 days after the first contact with allergens. Slow reactions usually appear 24–72 hours after re-exposure to allergen.[15] In this study, increase thickness of the ear lobe was seen in group B and C (Figure 1). Sensitization phase begins with the exposure hapten on the body, then bind with protein structural to form a hapten-carrier complex. Hapten-carrier complex is then recognized by the Langerhans cells which function as antigen presenting cells (APC). These Langerhans cells and their maturation then migrate to the lymph nodes, particularly to the lymph node, and presenting antigens to lymphocytes T.[18] Normally, sensitization phase occur in 10–14 days.[19,20] After antigen presented to the cells of $CD4^+$ T helper (Th) proliferation followed by clonal expansion to be antigen specific (memory) T cells.[21,23]

Tongue piercing may result in a trauma on the tongue,[21] which triggering the inflammatory response as an attempt of the body to maintain homeostasis under the influence of adverse environmental effect.[19] There was infiltration of mononuclear cells, monocytes and lymphocytes in the histological HE tissue slides of group B and group C. Mononuclear cell infiltration shown in group B were treated by the insertion of tongue piercing and the group C was treated with exposure to $HgCl_2$ (Figure 1, 3 and 4). The group treated by tongue piercing showed a reaction which is same with the group treated with $HgCl_2$. The results are consistent with previous research which showed infiltration of mononuclear cells (lymphocytes and macrophages) in the tongue piercing in rats, Sprague dawley up to 12 weeks,[14] and similar with the result of study in Beagles dogs.[17] In addition, similar results are also shown in this study in tongue piercing for 2 weeks. Lymphocytes and granulation tissue predominantly found in the area around the tongue piercing.[14,21] Exposure to $HgCl_2$ can induce the contacts hypersensitivity that also showed infiltration of mononuclear cells.[13] Mercury (Hg) is an alloy which is one component of amalgam in dentistry and Hg in the $HgCl_2$ is reported to be toxic.[22] Mercury is also a strong allergen and can induce polymorphonuclear cells and macrophages infiltration in rats [13]. It was related to the results of previous study which showed infiltration of mononuclear cells in rats tongue after 6 hours of re-exposure with tongue piercing. Repeated exposure of allergen directly recognized by T cell effectors and the cells release lymphokines as a signal for mononuclear cells in order to attract the cells to the exposed area to phagocyte the allergens. The initial symptoms of contact hypersensitivity can be seen 4-6 hours

after re-exposure, which showed mononuclear cells such as lymphocytes and monocytes out of the blood vessels and move between endothelial cells to the injury site. Mononuclear cells would be dominant in the area of injury that seen in the histological tissue slide.[18,19]

Type IV hypersensitivity is a slow reaction, which taking place in 24-48 hours. T lymphocytes provide receptors on the macrophages to bind the antigen. This resulted in sensitized T cells that will release lymphokine which acts as a mediator of delayed type hypersensitivity. Manifestations of this reaction are infiltration of monocytes and lymphocytes, or macrophages and cause tissue swelling at the site of antigen. The release of lymphokines by sensitized of T cells will cause the accumulation of large numbers of macrophages and cells epitheloid who will develop giant cells. Tissue damage further due to the cytotoxicity of macrophages and perhaps natural killer cells is activated by lymphokines or limphotoxin.[12,19] Reaction similar suggested caused swelling of the ear lobe and the infiltration of mononuclear cells in the tongue piercing, so the possible mechanism also occurs in hypersensitivity contacts of tongue piercing.

The result of ANOVA and LSD indicate that there were significant difference of the number of mononuclear cells in each treatment between groups ($p<0.05$). This is probably due the increase of duration the contact hypersensitivity reaches the maximum intensity. The manifestation of contact hypersensitivity can be seen microscopically through increased infiltration of mononuclear cells although sometimes not directly proportional to the clinical manifestation macroscopically[15,16] and this is accordance with the theory that the exposure of foreign materials in a long time can cause the cellular reaction of body which is dominated by mononuclear cells in the area of the injury.[14,19,23]

It was concluded that tongue piercing induce contact hypersensitivity in male Wistar rats *(Rattus norvegicus)*. It is characterized clinically by increasing the ear lobe thickness, histologically by the infiltration of monocyte and lymphocyte. Further research needed to determine cytokines in specific immune reaction that indicate the body's reaction to the tongue piercing.

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