Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/ 21431
DOI URL: http://dx.doi.org/10.21474/IJAR01/ 21431

ISSN NO. 2320-5407

RESEARCH ARTICLE

SCREENING OF METAL IONS AMONG PATIENTS WITH AUTOIMMUNE THYROID DISEASES – A PILOT STUDY

Zahid Shakoor[1], Adel Almogren[1], Rana Hasanato[1], Rim Sghiri[1], Usman Zahid Shakoor[2], Saba Zahid Shakoor[3], Muqadas Areej[2], Izah Zahid Shakoor[3], Imran Zahid Shakoor[2] and Ruhma Khan[3]

1. Department of Pathology, College of Medicine, King Saud University Medical City, Riyadh, Kingdom of Saudi Arabia
2. Shifa College of Medicine, Islamabad, Pakistan
3. Islamabad Medical and Dental College, Islamabad, Pakistan.

Manuscript Info

Manuscript History
Received: 19 May 2025
Final Accepted: 21 June 2025
Published: July 2025

Key words:-
Nickel sulfate, titanium, chromium, autoimmune, thyroid diseases.

Abstract

Background: Autoimmune thyroid diseases (AITDs) are organ specific disord ers of unknown etiology. Metal ions have been implicated in induction of autoi mmunity.

Methods: This analytical cross sectional pilotstudy of 100 patients with AITDs (73females and 27 males; mean age of 34.6±14.7 years) and 100 normal healthy individuals (mean age 38.1±9.4 years; 66 females and 34 males) was performed at King Khalid University Hospital, Riyadh. Serum nickel sulfate, titanium and chromium were measured by inductively coupled plasma-mass spectrometry (ICP-MS) instrument (Perkin-Elmer).

Results: Mean serum concentration of nickel sulfate among patients with AIT Ds (0.0068±0.002 µg/L;95% CI 0.006330-0.007410) was significantly higher than the normal controls (0.0059±0.001 µg/L;95% CI 0.005598-0.006288; p < 0.004). The mean serum levels of titanium among the patients with AITDs(0.05 3±0.09 µg/L; 95% CI0.5195-0.5567) and the control group (0.053±0.09 µg/L; 95% CI 0.5170-0.5538) were no different. Similarly, serum chromium levels among patients with AITDs (0.177±0.02 µg/L;95% CI 0.1723-0.1825) and controls (0.175±0.02 µg/L; 95% CI 0.1715-0.1813) were also devoid of any statistical significance. High serum level of nickel among the patients with AITDs did not correlate with anti-thyroid antibodies.

Introduction:-

Auto immune thyroid disease comprise of Graves' disease and chronic auto immune thyroiditis clinically manifestig as hyper and hypothyroidism respectively.[1] The pathological hallmark of thyroid autoimmunity is the intense lymphocytic infiltration and the presence of anti-thyroglobulin, anti-thyroid peroxidase and thyroid stimulating hormone receptor antibodies.[2] Whereas the precise mechanism of induction of autoimmunity remains obscure repeated exposure to metals has been implicated in immune mediated pathologies including allergic and autoimmune reactions.[3] The capacity of metals to trigger autoimmune reactions is based upon the ability of some metals to induce MHC-restricted T cell activation against self-peptides by establishing non-covalent bridging between self-peptide-major histocompatibility complex (MHC) and T cell receptor.

Corresponding Author:-Zahid Shakoor
Address:- Professor, Department of Pathology, College of Medicine, King Saud University Medical City, Riyadh, Kingdom of Saudi Arabia.

Nickel is a ubiquitous metal and is the principle cause of allergic contact dermatitis along with other less frequently implicated metals such as cobalt, gold and chromium.[5] Data linking nickel with autoimmunity are lacking. Experimental studies however have revealed that subsequent to administration of nickel either by oral or subcutaneous routes Norway rats develop autoimmunity with the production anti-nuclear antibodies.[6] Similarly, data regarding titanium induced inflammation are scarce. There is however one report describing titanium induced hypersensitivity reaction following placement of an occlusive device for patent foramen ovale.[7] The nature of contact, the length and the route of exposure along with the dose of the nickel or other metal ions may be the main determinants for induction of autoimmunity in a susceptible individual. This study was performed to assess the blood levels of nickel, titanium and chromium among patients with AITDs.

Patients and methods

This analytical cross-sectional study was performed in the clinical chemistry unit at King Khalid University Hospital, Riyadh between March 2019 and May 2020. A total of 100 patients with thyroid autoimmune diseases harboring anti-thyroid antibodies were enrolled in the study. Patients were included in the study on the basis of presence of anti-thyroid antibodies, ultrasonographic and histological evidence for autoimmune diseases. Details about the presenting signs and symptoms could not be obtained because of lack of access to patient records. This, group of patients included 73 females and 27 males with the mean age of 34.6 $\pm$ 14.7 years. A group of 100 healthy controls was also recruited from the members of hospital staff and blood bank donors. This group comprised of 66 females and 34 males (mean age 38.1 $\pm$ 9.4 years) with no serological evidence of thyroid autoimmunity (Table 1).

Since there was no previous report investigating nickel association with AITDs the sample size was chosen arbitrarily to perform this pilot study. Based on the thyroid hormone profiles patients with thyroid autoimmunity were grouped into five categories. Majority of the patients (48; 48%) had low thyroid stimulating hormone (TSH) with normal free thyroxin (fT4) and were categorized as sub-clinical hyperthyroidism. There were 39 (39%) patients with sub-clinical hypothyroidism with high TSH and normal fT4 blood levels. Chronic autoimmune thyroiditis group comprised of 8 (8%) patients with high TSH and low fT4 blood levels. Graves' disease group with high levels of fT4 and low blood levels of TSH included 5 (5%) patients. Euthyroid status with normal levels of TSH and fT4was found in 43 (43%) AITD patients (Table 2).

Anti-thyroid antibodies both anti-thyroid peroxidase (TPO) and anti-thyroglobulin (TG) were assessed by enzyme-linked immunosorbent assay (ELISA) using Quanta Lite kits (Inova diagnostics, Ingbert, Germany). For the interpretation of results cut off value for anti-TPO antibodies was 100 WHO units and for anti-TG antibodies was 60 WHO units. Thyroid hormones were assessed by electrochemiluminescence assay using Cobas e 411 analyzer (Roche diagnostics, Mannheim, Germany). The normal range for TSH was 0.25 to 5mIU/L and 12 to 22pmol/Lfor fT4. After obtaining informed consent 5 ml of venous blood was collected both from patients and controls in a plain vacutainer. Each sample was allowed to clot and serum was extracted after centrifugation. Serum concentrations of nickel, titanium and chromium were measured by inductively coupled plasma-mass spectrometry (ICP-MS) instrument (Perkin-Elmer). This study was approved by the institutional review board of the College of Medicine.

Statistical analysis:

Data were analyzed by MedCalc computer software version 14.8.1. Normally distributed numeric data were summarized as mean $\pm$ standard deviation, non-normally distributed data were expressed as median and interquartile range and categorical data were summarized as numbers and percentages. Comparison between the groups was performed using *t* test and a $p < 0.05$ was considered statistically significant.

Results:-

Fig. 1 describes comparison of serum levels of nickel sulfate between patients with AITDs and normal controls. The mean serum level of nickel sulfate among patients with AITDs was 0.0068 $\pm$ 0.002μg/L (95% CI 0.006330 to 0.007410) whereas among the controls the mean serum level of nickel sulfate was 0.0059 $\pm$ 0.001 μg/L (95% CI 0.005598 to 0.006288). Comparative analysis revealed that patients with AITDs harbored significantly higher levels of nickel sulfate in their blood compared to the normal healthy controls ($p < 0.004$). The mean serum levels of titanium among the patients with AITDs (0.053 $\pm$ 0.09 μg/L; 95% CI0.5195 to 0.5567) and the normal healthy controls (0.053 $\pm$ 0.09 μg/L; 95% CI 0.5170 to 0.5538) were no different (Fig. 2). Similarly, serum level of chromium among the patients with AITDs was 0.177 $\pm$ 0.02 μg/L (95% CI 0.1723 to 0.1825) and among the healthy controls was 0.175 $\pm$ 0.02μg/L (95% CI 0.1715 to 0.1813). The difference between the two groups was devoid of statistical significance (Fig. 3). In order to assess if the elevated serum levels of nickel sulfate had any relationship with the

concentration of anti-thyroid antibodies, correlations were sought between serum nickel and anti-TPO and anti-TG antibody concentrations. The serum concentration of nickel sulfate did not correlate either with anti-TPO or anti-TG antibodies.

Discussion:-

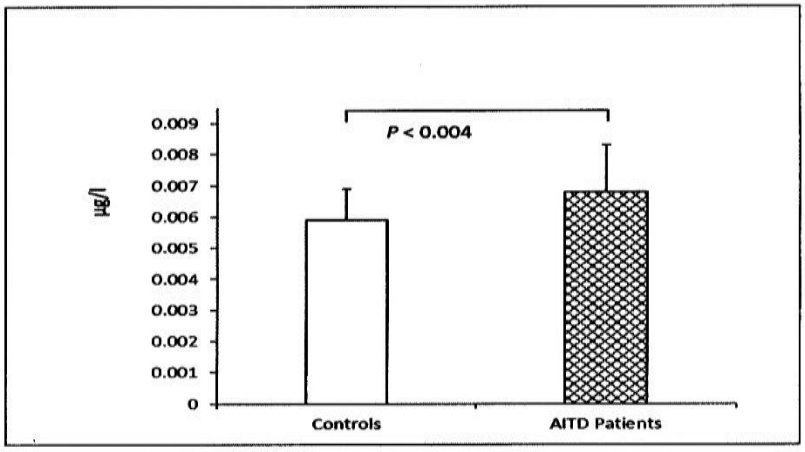
Among the metal ions investigated in the present study blood level of nickel sulfate was significantly higher among patients with AITDs. Data linking nickel with development of AITDs are lacking. However, metals as haptens have been implicated in various immune processes and inflammation.[8] Systemic nickel allergy syndrome (SNAS)has recently been described as a chronic inflammatory disorder and has been categorized as a distinct entity.[9] It occurs in about 20% of patients with nickel allergy and is characterized by eczema, systemic contact dermatitis along with other manifestations involving gastrointestinal, neurological and respiratory systems.[10] Although the exact mechanism of inflammation in SNAS remains obscure a mixed Th1 and Th2 immune responses have been described in the pathogenesis of SNAS.[11] It has recently been observed that chronic autoimmune thyroiditis tends to occur two folds more frequently among patients with SNAS compare to occurrence of chronic autoimmune thyroiditis among patients suffering from other autoimmune disorders.[12] It is therefore quite conceivable that nickel plays a pivotal role in SNAS and may also be involved in induction of thyroid autoimmunity by enhancing thyroid antigenicity and promoting autoimmune responses.

Nickel sulfate is widely distributed in the environment and human exposure may occur by inhalation in electroplating industry[13], ingestion of vegetables, fruits, and cereal crops grown in contaminated areas[14], physical contact in the form of wearing nickel earrings, piercing jewelry[15] keys[16] laptop computers[17], children toys[18] and release from nickel containing dental restorative materials.[19] After gaining access into human body nickel binds to serum albumin forming a human serum albumin-nickel complex in the vicinity of T cell receptor and antigen presenting cell expressed MHC molecules thus facilitating nickel delivery.[20]Binding of nickel may alter self-antigens that may trigger presentation of MHC embedded self-peptide resulting in activation T lymphocytes against self-peptides and induction of autoimmunity.[21]Existence of nickel specific T cells have already been described among patients with contact allergy to nickel[22] and itis possible that this subset of T lymphocytes may be involved in autoimmune responses. In addition, one of the most debilitating nickel induced affliction is induction of apoptosis[23] that may also be critical for tissue damage associated with AITDs.Nickel along with other metals such as mercury and gold elicits delayed type of hypersensitivity reaction and has been implicated in the pathogenesis of rheumatoid arthritis, systemic lupus erythematosus and Sjögren's syndrome[24] suggesting a possible role of the metal ions in induction of systemic autoimmunity.

Serum levels of titanium and chromium between the patients with AITDs and controls were no different in this study. Insertion of nickel–titanium chin implant for cosmetic reasons in females has been shown to induce systemic autoimmune syndrome known as Kikuchi–Fujimoto syndrome mimicking clinical features of systemic lupus erythematosus, adult onset Still's disease, anti-phospholipid syndrome, and hemophagocytic syndrome.[25] It is possible that exposure to nickel and titanium in conjunction may have enhanced the antigenic nature of self-antigens to trigger autoimmune responses. In addition, an experimental study investigating long-term exposure to titanium in mice has demonstrated that titanium dioxide induced inflammatory responses are mediated by activation of NFκB.[26] Similarly, it has been observed among patients undergoing fixed orthodontic treatment that increasing concentrations of nickel and chromium in gingival crevicular fluid are associated with severity of inflammatory changes in the gingival tissues.[27] Despite no elevation in titanium and chromium levels these metals as haptens may contribute in inflammatory responses in conjunction with higher concentration of nickel ions. Metal ions by altering the antigenic characteristics of self-peptides along with their potential to induce cytokines may be involved in induction of autoimmunity. AITDs are single organ autoimmune diseases and it is difficult to exclude the presence of local predisposition in terms of genetic factors in the pathogenesis of AITDs.

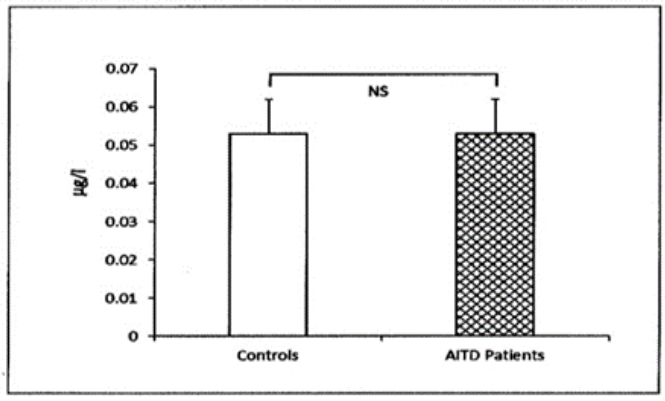
In conclusion, patients with AITDs were found to have significantly higher concentration of serum nickel sulfate suggesting an association between nickel and thyroid autoimmunity. Data describing role of nickel sulfate in the induction of autoimmune diseases are scarce and the findings of this study require validation in large scale studies. Furthermore, there appears to be a need of investigation for identification of local predisposing factors in the thyroid tissue among susceptible individuals for induction of thyroid autoimmunity.

Fig 1. Comparison of serum levels of nickel sulfate between patients with autoimmune thyroid diseases and normal healthy controls



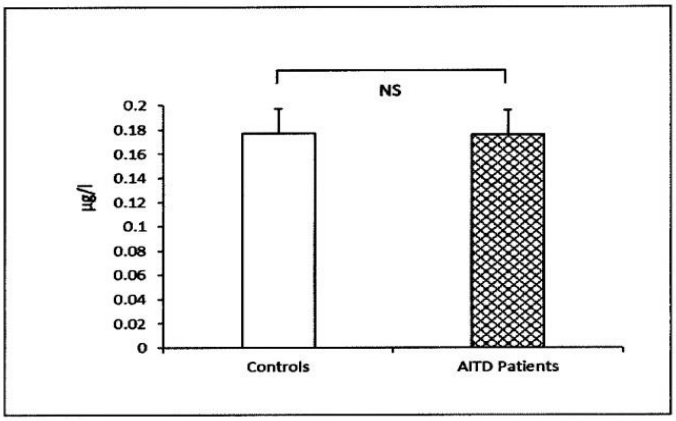
Controls: n = 100, Autoimmune thyroid disease (AITD) patients: n = 100

Fig 2. Comparison of serum levels of titanium between patients with autoimmune thyroid diseases and normal healthy controls



Controls: n = 100, Autoimmune thyroid disease (AITD) patients: n = 100, NS = Not significant

Fig 3. Comparison of serum levels of chromium between patients with autoimmune thyroid diseases and normal healthy controls



Controls: n = 100, Autoimmune thyroid disease (AITD) patients: n = 100, NS = Not significant

Table 1. Characteristic features of patients with autoimmune thyroid disease and normal healthy controls.

Variable	Patients	Controls
Number	100	100
Males n (%)	27 (27%)	34 (34%)
Females	73 (73%)	66 (66%)
Mean age ±sd years	34.6 ± 14.7	38.1 ± 9.4
TG antibodies	67%	ND
TPO antibodies	93%	ND
Coexisting TG and TPO	55%	-

TPO = Thyroid peroxidase, TG = Thyroglobulin, ND = Not detected, sd = Standard deviation

Table2. Categories of patients with autoimmune thyroid diseases based upon thyroid hormone profile and anti-thyroid antibodies.

Condition	n (%)	TSH NR 0.25-5mIU/L	fT4 NR 12-22 pmol/L	TG Abs >60 WHO Units	TPO Abs >100 WHO Units
GD	5 (5%)	0.05 (0.045)	28.14 (34.96)	154.2 (383.3)	218.8 (1370)
SH	5 (5%)	0.14 (0.265)	21.4 (8.42)	141.7 (476.3)	795.7 (732.5)
CAT	8 (8%)	26.93 (97.62)	10.68 (7.19)	734.9 (1133.5)	1593.5 (1788.5)
SAH	39 (39%)	9.13 (8.62)	15 (3.5)	164 (433.12)	756.42 (1298.3)
ET	43 (43%)	2.27 (1.14)	15.5 (3.86)	218.5 (714.3)	536.9 (1026)

Each value is represented as median and interquartile range, GD = Graves' disease, SH = Subclinical hyperthyroidism, CAT = Chronic autoimmune thyroiditis, SAH = Subclinical autoimmune hypothyroidism, ET = Euthyroid. TG Abs = Thyroglobulin antibodies, TPO Abs = Thyroid peroxidase antibody, TSH = Thyroid stimulating hormone, fT4 = Free T4, n = Number, NR = Normal range

Acknowledgement:-

The authors would like to thank Mr. Mohammad Attya for his assistance for estimation of serum levels of metals using inductively coupled plasma-mass spectrometry (ICP-MS) instrument.

References::-

1. Collins J, Gough S. Autoimmunity in thyroid disease. Eur J Nucl Med Mol Imaging 2002; 29: 417-424.
2. Nielsen CH, Hegedüs L, Leslie RG. Autoantibodies in autoimmune thyroid diseasepromote immune complex formation with self-antigens and increase B cell and CD4+ T cellproliferation in response to self-antigens. Eur J Immunol 2004; 34: 263-272.
3. Weltzien HU, Moulon C, Martin S,Padovan E, Hartmann U, Kohler J. T cell immune responses tohaptens. Structural models for allergic and autoimmune reactions.Toxicology. 1996; 107:141–151.
4. Louis-Dit-Sully C, Schamel WW. Activation of the TCR complex by small chemical compounds. EXS. 2014;104:25-39. doi: 10.1007/978-3-0348-0726-5_3.
5. Thyssen JP, Menne´ T. Metal allergy—a review on exposures,penetration, genetics, prevalence, and clinical implications.Chem Res Toxicol. 2010; 23:309.
6. Al-Mogairen SM. Induction of autoimmunity in brown Norway rats by oral and parenteral administration of nickel chloride. Lupus. 2010;19(3):262-7. doi: 10.1177/0961203309351728. Epub 2009 Dec 14.
7. Fukahara K, Minami K, Reiss N, Fassbender D, Koerfer R. Systemic allergic reaction to the percutaneous patent foramen ovaleoccluder. J Thorac Cardiovasc Surg 2003;125:213–214
8. L.H. Duntas, Environmental factors and autoimmune thyroiditis.Nat. Clin. Pract. Endocrinol. Metab. 2008; 4, 454–460.
9. Cirla AM, Cirla PE. Nickel dermatitis, systemic nickel allergy syndrome, immuno-genesis, immune-tolerance: an Italian study. G. Ital. Med. Lav. Ergon. 2012; 34, 147–149
10. Ricciardi L, Arena A, Arena E, Zambito M, Ingrassia A, Valenti G, et al. Systemic nickelallergy syndrome: epidemiological data from four Italian allergyunits. Int. J. Immunopathol. Pharmacol. 2014; 27:131–136.
11. Minelli M, Schiavino D, Musca F, Bruno ME, Falagiani P, Mistrello G, et al. Oralhyposensitizationtonickel induces clinical improvement and a decrease in TH1 andTH2 cytokines in patients with systemic nickel allergy syndrome.Int. J. Immunopathol. Pharmacol. 2010; 23: 193–201
12. Andrioli M, Trimboli P, Maio D, Persani L, Minelli M. Systemic nickel allergic syndrome as an immune-mediated disease with an increased risk for thyroid autoimmunity. Endocrine. 2015 ;50(3):807-10. doi: 10.1007/s12020-015-0581-2. Epub 2015 Mar 21.

13. Tsao YC, Chang YC, Gu PW, Tzeng IS, Luo JJ. Characteristics of Decreased Pulmonary Function and Clinical Symptoms in Nickel Electroplating Workers. J Occup Environ Med. 2017 Oct 23. doi: 10.1097/JOM.0000000000001204. [Epub ahead of print]
14. Nawab J, Farooqi S, Xiaoping W, Khan S, Khan A. Levels, dietary intake, and health risk of potentially toxic metals in vegetables, fruits, and cereal crops in Pakistan. Environ Sci Pollut Res Int. 2017 Dec 8. doi: 10.1007/s11356-017-0764-x. [Epub ahead of print]
15. Suzuki NM, Duarte IAG, Hafner MFS, Lazzarini R. Nickel exposure from keys: a Brazilian issue. An Bras Dermatol. 2017; 92(5): 734-735. doi: 10.1590/abd1806-4841.20175754.
16. Midander K, Hurtig A, Borg Tornberg A, Julander A. Allergy risks with laptop computers - nickel and cobalt release. Contact Dermatitis. 2016; 74(6): 353-9. doi: 10.1111/cod.12525. Epub 2016 Jan 19
17. Uter W, Wolter J. Nickel and cobalt release from earrings and piercing jewelry - analytical results of a German survey in 2014. Contact Dermatitis. 2017 Dec 28. doi: 10.1111/cod.12941. [Epub ahead of print]
18. Overgaard LE, Engebretsen KA, Jensen P, Johansen JD, Thyssen JP. Nickel released from children's toys is deposited on the skin. Contact Dermatitis. 2016 Jun;74(6):380-1. doi: 10.1111/cod.12553.
19. Bjørklund G, Dadar M, Aaseth J. Delayed-type hypersensitivity to metals in connective tissue diseases and fibromyalgia. Environ Res. 2018; 161: 573-579. doi: 10.1016/j.envres.2017.12.004.
20. Thierse HJ, Moulon C, Allespach Y, Zimmermann B, Doetze A, Kuppig S, et al. Metal-protein complex-mediated transport and delivery of Ni2+ to TCR/MHC contact sites in nickel-specific human T cell activation. J Immunol. 2004;172(3):1926-34.
21. Griem P, Vonvultee C, Panthel K, Best SL, Sadler PJ, Shaw CF. 1998. T-cell cross-reactivity to heavy-metalsidentical cryptic peptides may be presented from protein exposed to different metals. Eur. J. Immunol. 1998; 28:1941–1947.
22. Kapsenberg ML, Res P, Bos JD, Schootemijer A, Teunissen MB, Van Schooten W. Nickel-specific T lymphocyte clones derived from allergic nickel-contact dermatitis lesions in man: heterogeneity based on requirement of dendritic antigen-presenting cell subsets. Eur J Immunol. 1987;17(6):861-5.
23. Rana SV. Metals and apoptosis: recent developments. J Trace Elem Med Biol. 2008;22(4):262-84. doi: 10.1016/j.jtemb.2008.08.002. Epub 2008 Oct 10
24. Stejskal V, Reynolds T, Bjørklund G. Increased frequency of delayed type hypersensitivity to metals in patients with connective tissue disease. J Trace Elem Med Biol. 2015;31:230-6. doi: 10.1016/j.jtemb.2015.01.001. Epub 2015 Jan 14.
25. Loyo E, Jara LJ, López PD, Puig AC. Autoimmunity in connection with a metal implant: a case of autoimmune/autoinflammatory syndrome induced by adjuvants. Auto Immun Highlights. 2013; 4(1): 33–38.
26. Liu D, Zhou JL, Hong F, Zhang YQ. Lung inflammation caused by long-term exposure to titanium dioxide in mice involving in NF-κB signaling pathway. J Biomed Mater Res A. 2017;105(3):720-727. doi: 10.1002/jbm.a.35945. Epub 2016 Nov 7.
27. Bhasin V, Pustake SJ, Joshi V, Tiwari A, Bhasin M, Punia RS. Assessment of Changes in Nickel and Chromium Levels in the Gingival Crevicular Fluid during Fixed Orthodontic Treatment. J Contemp Dent Pract. 2017;18(8):675-678.