

Estimation of major immunoglobulins in smokers and gutkha chewers

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Abstract

Aim: To estimate the level of IgG and IgA major immunoglobulins in patients having the habit of smoking, gutkha chewing and in patients without any tobacco habit as control.

Materials and Methods: Estimation of major immunoglobulins IgG and IgA was carried out by automated Nephelometry method in ten patients (control group), forty patients who had habit of smoking either bidi or cigarette and forty patients who had the habit of gutkha chewing. Among forty patients who smoked, twenty patients were without any lesion while twenty patients had homogenous leukoplakia. Among the forty patients who had habit of gutkha chewing, twenty patients were without any lesion while twenty patients had oral submucous fibrosis (OSMF). The obtained data were analyzed using independent sample *t*-test.

Results: IgG and IgA levels were higher in smokers and gutkha chewers as compared to control group and were higher in gutkha chewers as compared to smokers. IgG and IgA levels of non-lesional smokers and gutkha chewers showed no change as compared to the controls while it was increased in patients with homogenous leukoplakia and patients with OSMF as compared to control group. IgG and IgA levels were also significantly higher in patients with OSMF as compared to that of homogenous leukoplakia. IgG and IgA levels were higher in all the grades of OSMF as compared to the controls and both IgG and IgA levels were directly correlated with the grades of OSMF.

Conclusion: Higher major immunoglobulins levels in present study among the study groups indicate the use of immunoprofile estimation in etiology and pathogenesis and would prove a great asset in the proper assessment of the lesions.

Key Words: Gutkha chewers, homogenous leukoplakia, IgG, IgA, oral submucous fibrosis, smokers

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INTRODUCTION

The effects of tobacco on the oral tissues have been an area of interest to researchers for a long time. Tobacco habit encountered around the world is mainly in the form of tobacco smoking, tobacco chewing and tobacco snuff use.^[1]

Tobacco smoking is an important risk factor for precancerous lesion of mouth. Smokers have a significantly high prevalence of Leukoplakia.^[2] Leukoplakia is currently defined as “a white patch or plaque that cannot be characterized clinically or pathologically as any other disease”. Tobacco smoke affects multiple organ system but especially relates to the respiratory

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tract and the cardiovascular system. It influences the immune system in many ways.^[3] Tobacco smoking affects a wide range of immunological functions in human and experimental animals including both humoral and cell mediated immune responses.^[4] According to Frial, nicotine activates dendritic cell and augments their capacity to stimulate T cell proliferation and cytokine secretion.^[4] The increasing use of pan masala/gutkha, a mixture of tobacco and a less moist form of betel quid lacking the betel leaf, seems to be associated with an earlier age of onset of oral submucous fibrosis (OSMF). It is a chronic debilitating disease of oral cavity associated with arecanut (betel-nut) chewing, other factors are believed to contribute to the development of OSF, including general nutritional and vitamin deficiencies and hypersensitivity to certain dietary constituents such as chilli peppers and chewing tobacco.^[5] It affects all parts of oral mucosa and oro-nasopharynx.^[6] According to Pindborg, OSMF is defined “as an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxta-epithelial inflammatory reaction followed by a fibro-elastic change of the lamina propria with epithelial atrophy leading to stiffness of mucosa causing trismus and inability to eat”.^[7] The etiology of OSMF is considered to be multifactorial, arecanut being the primary etiology.^[8] However, occurrences of OSMF in cases without any history of using irritants as seen in patients with OSMF, have been noted that may be due to idiopathic or autoimmune nature of disease.^[9] Hyperimmunoglobulinemia is invariably associated with OSMF.^[10] Therefore, Patidar *et al.* had assessed serum immunoglobulin levels in OSMF patients to define the status of humoral immunity.^[9] Tobacco in any form impairs immune function of the body that is carried out by immunoglobulin.

Immunoglobulins are synthesized by plasma cells and to some extent by lymphocytes. Five classes of immunoglobulins have been recognized – IgG, IgA, IgM, IgD, and IgE (WHO 1964) from them IgG, IgA and IgM are major and IgD and IgE are minor immunoglobulins.^[11] The present study was carried out to assess the level of major immunoglobulins IgG and IgA in smokers and gutkha chewers with regards to causation and advancement of the disease occurring due to smoking and gutkha chewing habit.

MATERIALS AND METHODS

The present study comprised ninety patients who had attended the Government Dental College and Hospital during the period of 2011-2013. Of the ninety patients, ten patients were considered under control group; forty patients had habit of smoking either bidi or cigarette, of them twenty patients were without any lesion while twenty patients had homogenous

leukoplakia; and forty patients had the habit of gutkha chewing of them twenty patients were without any lesion while twenty patients had OSMF. Detailed clinical history of each patient was recorded. Patients with diabetes mellitus, liver disorder, endocrine disorders, hypertension, myocardial infarction, stroke, thyroid, any respiratory infection, allergic conditions and autoimmune diseases were excluded. Patients with habit of tobacco smoking and gutkha chewing without any lesion, patients with homogenous leukoplakia and OSMF and control group without any habit and lesion and all lesional patients without dysplasia were included.

Patients were divided into following groups according to the clinical findings:

- Group I: Ten patients as control group
- Group II: Twenty patients with habit of smoking without any lesion
- Group III: Twenty patients of homogenous leukoplakia [Figure 1]
- Group IV: Twenty patients with habit of gutkha chewing without any lesion
- Group V: Twenty patients of OSMF [Figure 2].

The OSMF patients were clinically graded according to Khanna JN, Dave R (1995) as:

Grade I

Mouth opening 35-40 mm. Very early or incipient stage burning sensation; dryness of mouth; vesicles or ulcerations; irritation with spicy food; no change in mucosal color; no fibrous bands palpable and tongue protrusion normal.

Grade II

Mouth opening 25-35 mm. Early stage Burning sensation and dryness of mouth, irritation with spicy food; oral mucosa blanched and loss of elasticity; no clear cut fibrotic band; slight restriction of mouth opening and tongue protrusion normal.



Figure 1: Clinical photograph showing homogenous leukoplakia

Grade III

Mouth opening 15-25 mm. Moderately advanced stage Burning sensation and dryness of mouth; irritation with spicy food; blanched, opaque, leather-like mucosa; vertical fibrotic bands on buccal mucosa making it stiff; considerable restriction of mouth opening; tongue protrusion not much affected; difficulty in eating and speaking and poor oral hygiene.

Grade IV

Mouth opening <15 mm. Advanced stage Burning sensation and dryness of mouth; irritation with spicy food; blanched, opaque, leather-like mucosa; thick fibrous bands on both buccal mucosa, retromolar area and at pterygomandibular raphe; very little mouth opening; restricted tongue protrusion; impaired speech and eating and very poor oral hygiene.

Serum immunoglobulins estimation

About 2-3 ml blood was collected for serum immunoglobulins estimation from antecubital vein. Separated serum was collected in separate container and serum level of IgG and IgA was estimated by automated Nephelometry method.

Histopathological study

Incisional biopsy was performed from the lesions of homogenous leukoplakia and OSMF of the patients under 2% lignocaine and excised tissues were used for histopathologic examination [Figures 3 and 4].

RESULTS

Clinical grades of OSMF were correlated with histopathological grades. IgG and IgA levels were higher in smokers and gutkha chewers as compared to control and were higher in gutkha chewers as compared to smokers but non significant. IgG and IgA levels were higher in gutkha chewers with OSMF than control which was statistically significant (P values for IgG and IgA were 0.013 and 0.037 respectively) but when comparison was made between gutkha chewers without any lesion and OSMF, it was higher in OSMF and the difference was statistically highly significant. (P values for IgG and IgA were 0.001 and 0.01 respectively). IgG and IgA levels were higher in OSMF than homogenous leukoplakia which was statistically significant. (P values for IgG and IgA were 0.013 and 0.024 respectively) [Table 1]. IgG and IgA levels were significantly ($P < 0.01$) increased in the all grades of OSMF as compared to control and both IgG and IgA levels directly correlated with the grades of OSMF [Table 2].

DISCUSSION

There were reports in literature that tobacco in any form impairs immune function of the body that is carried out by immunoglobulin. It has been demonstrated that



Figure 2: Clinical photograph showing grade III OSMF

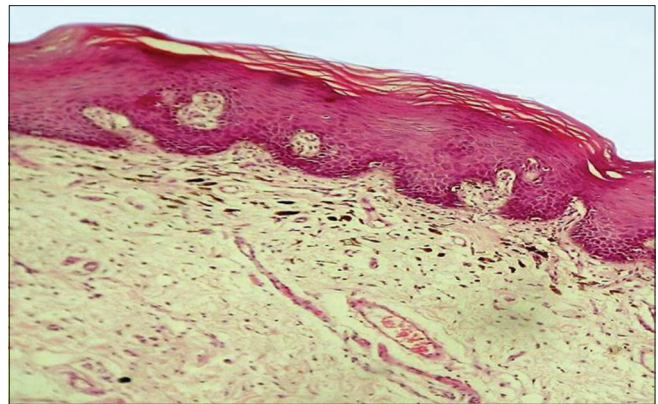


Figure 3: Photomicrograph of homogenous leukoplakia showing hyperkeratosis (H&E stain, $\times 100$)

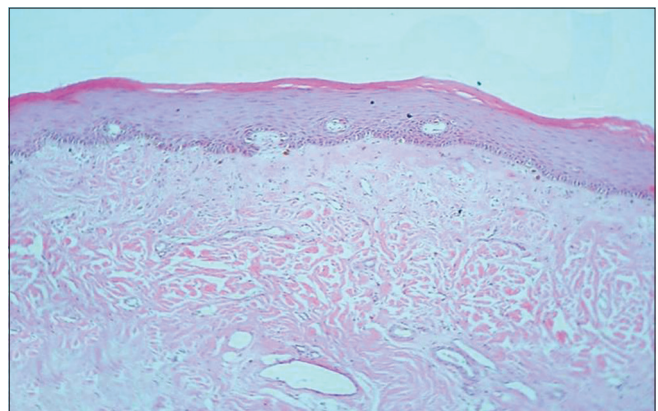


Figure 4: Photomicrograph of grade III OSMF showing hyperkeratinized atrophic epithelium with underlying connective tissue showing hyalinization of collagen (H&E stain, $\times 100$)

mucosal immunity is depressed among tobacco smokers and chewers.^[1] In animal study, it is noticed that chronic exposure to nicotine, one of the major components of tobacco, causes immunosuppression by impairment of antigen mediated signaling in T cells.^[4] IgG and IgA levels were higher in smokers and gutkha chewers as compared to control and were higher in gutkha chewers as compared to smokers. This observation reflects the response of the immune system to irritation of both

Table 1: Comparison of major immunoglobulin levels of control with lesional and nonlesional smokers and gutkha chewers

Study group	Major immunoglobulins (Mean±SD)	
	IgG	IgA
Control	11.13±1.32	1.81±0.79
Nonlesional smoker	11.69±2.07	1.82±0.70
Homogenous leukoplakia	12.57±2.40	2.01±0.51
Nonlesional gutkha chewers	11.67±0.91	1.90±0.59
OSMF	13.83±2.03	2.40±0.39
Statistical analysis	P	
	IgG	IgA
Independent sample <i>t</i> -test between control and non lesional smokers	0.579	0.971
Independent sample <i>t</i> -test between control and homogenous leukoplakia	0.225	0.516
Independent sample <i>t</i> -test between control and nonlesional gutkha chewers	0.319	0.801
Independent sample <i>t</i> -test between control and OSMF	0.013	0.037
Independent sample <i>t</i> -test between nonlesional smokers and homogenous leukoplakia	0.296	0.414
Independent sample <i>t</i> -test between nonlesional gutkha chewers and OSMF	0.001	0.01
Independent sample <i>t</i> -test between homogenous leukoplakia and OSMF	0.013	0.024

IgG: Immunoglobulin G, IgA: Immunoglobulin A, SD: Standard deviation, OSMF: Oral submucous fibrosis

Table 2: Comparison of major immunoglobulin levels with control and different grades of OSMF

Study group	Mean±SD	
	IgG	IgA
Control	11.13±1.32	1.81±0.79
Grade I	11.38±0.41	2.14±0.34
Grade II	12.60±0.42	2.33±0.29
Grade III	14.23±0.57	2.49±0.64
Grade IV	16.52±0.84	2.59±0.13
Statistical analysis	P	
	IgG	IgA
Independent sample <i>t</i> -test between Grade I and control	0.771	0.531
Independent sample <i>t</i> -test between Grade II and control	0.072	0.259
Independent sample <i>t</i> -test between Grade III and control	0.003	0.206
Independent sample <i>t</i> -test between Grade IV and control	<0.0001	0.097
Independent sample <i>t</i> -test between Grade I and Grade II	0.012	0.459
Independent sample <i>t</i> -test between Grade I and Grade III	0.001	0.428
Independent sample <i>t</i> -test between Grade I and Grade IV	<0.0001	0.056
Independent sample <i>t</i> -test between Grade II and Grade III	0.004	0.654
Independent sample <i>t</i> -test between Grade II and Grade IV	<0.0001	0.151
Independent sample <i>t</i> -test between Grade III and Grade IV	0.004	0.778

IgG: Immunoglobulin G, IgA: Immunoglobulin A, SD: Standard deviation, OSMF: Oral submucous fibrosis

the respiratory and gastrointestinal mucosa by cigarette/bidi smoke as IgA has been associated with seromucous membranes. IgA protects these membranes against myriads of soluble antigens by inhibiting their adherence to surface of mucosal cells. Thus the presence of tobacco smoke on these membranes results in increased production of this immunoglobulin.^[12] The higher mean IgG level in test subjects compared with controls also reflects a degree of secondary infection since IgG is the principal antibody in secondary antibody response. It may be suggested that continuous exposure to components of cigarette has stimulatory effects on immunoglobulin production, thus the increased levels of immunoglobulins.^[13] The raised levels of IgG in smokers might be one of the mechanisms to neutralize components of cigarette tobacco via complement activation.^[14] Serum IgG was significantly increased in OSMF patients as compared to control which are in agreement with the findings of Chaturvedi *et al.*,^[15] Pinakapani *et al.*^[6] and Patidar *et al.*^[9] but are not in agreement with the finding of Rajendran *et al.*^[16] Mean IgA level has been significantly increased in OSMF as compared to control which is similar to the observation made by Pinakapani *et al.*^[6] and Patidar *et al.*^[9] but is not in agreement with Chaturvedi and Marathe,^[17] Canniff *et al.*^[18] and Chaturvedi *et al.*^[15] Major immunoglobulins, i.e., IgG and IgA levels were increased significantly in OSMF than non-lesional gutkha chewers. Major immunoglobulins levels were significantly increased in patients with OSMF as compared to patients with homogenous leukoplakia. This finding is somewhat correlates with the finding of Rajendran *et al.*^[16] The possible mechanism behind increased levels of immunoglobulins may be that tobacco impairs the immunologic functions of the body carried out by immunoglobulins. In the tobacco habituals, the toxins are liberated by tobacco from bidi/cigarette smoke and arecolin from gutkha. The role of IgG in the body is to neutralize such toxins therefore there is increase the in the level of IgG in such patients.^[19] IgA in saliva is called secretory IgA and in serum it is called serum IgA. Because of the local irritants such as smoke or gutkha chewing, the level of secretory IgA is increased and by back diffusion into the serum, serum IgA levels are increased.^[9] Mean serum IgG levels were increased in all the grades of OSMF as compared to control group. The increase in serum IgG level was statistically highly significant in grade III and grade IV as compared to control. These findings are similar to those reported by Chaturvedi *et al.*^[15] Pinakapani *et al.*^[6] and Patidar *et al.*^[9] A highly significant increase in serum IgG level was also observed in grade I versus II, grade I versus III, grade I versus IV, grade II versus III, grade II versus IV and grade III versus IV cases of OSMF. These observations are quite similar to those reported by Pinakapani *et al.*^[6] and Patidar *et al.*^[9] but in contrast to Chaturvedi and Marathe.^[17] However, the difference in the levels of IgG in grade III was

significantly increased as compared to grade I. Also there was an increase in mean serum IgG according to increase in the grades of OSMF, which was correlating with findings of Pinakapani *et al.*^[6] and Patidar *et al.*^[9] In the present study the mean serum IgA level was subsequently increased in all the grades of OSMF as compared to control but not significantly. This finding is somewhat correlating with the findings of Pinakapani *et al.*^[6] and Patidar *et al.*^[9] but somewhat contrasting to the findings reported by Chaturvedi and Marathe.^[17] In the present study when comparison was made between the grades of OSMF accordingly grade I versus II, grade I versus III, grade I versus IV, grade II versus III, grade II versus IV and grade III versus IV, it was observed that there was a non-significant increase in serum IgA levels according to the grades of OSMF which was also reported by Pinakapani *et al.*^[6] but quite contrasting to the findings reported by Patidar *et al.*^[9] The increased levels of the serum IgG and IgA fractions of immunoglobulins in the study group patients highlight the role of active immune phenomenon at work in OSMF. The significant increase in levels of these major immunoglobulins is also suggestive of accelerated body defense at work among such patients. The elevated levels of IgG and IgA immunoglobulins are also in favor of polygammopathy, which are non-specific and non-diagnostic objective reflections of an underlying disease and aids in the clinical course of disease. Increase in major immunoglobulin levels is typically associated with three main chronic disease classes: Those affecting the liver, collagen and chronic infections. In the present study patients tested are free from any chronic infection or liver problems and have OSMF where there is deposition of collagen in oral tissue.^[20] It is clearly multifactorial disease and seems that the patients have a genetic predisposition which renders their oral mucosa susceptible to chronic inflammatory changes caused by chewing gutkha. In the present study, it is observed that the severity of OSMF was directly proportional to the estimated elevated levels of the major immunoglobulins IgG and IgA which may be taken as an indicator of the gravity of this oral condition and its management.^[20] A need is also felt for the knowledge of immunoprofile estimation in pathogenesis of such oral lesions that would prove a great asset in the proper assessment and management. From the findings related to the alteration in the immune profile of smokers and gutkha chewers, it can be suggested that there is definite role of immunity in occurrence of lesions in such patients. Therefore immune profile is beneficial in the early detection, prognosis and management of both the group of patients in the mass screening program as tobacco smoking and gutkha chewing habits are more prevalent now a days.

CONCLUSION

Immune profile is beneficial in early detection, management and prognosis of both the groups of patients in mass screening

program as tobacco smoking and gutkha chewing habits are more prevalent at present. Research into immunological aspects is becoming an excellent model for studying genetic-environmental-immunologic-nutritional interactions in disease pathogenesis.

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Conflicts of interest

There are no conflicts of interest.

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