



## RESEARCH ARTICLE

### ASSESSMENT BETWEEN STRESS, SERUM IMMUNOGLOBULIN A (IgA), GINGIVAL STATUS AND TEMPOROMANDIBULAR JOINT DISORDER AMONGST CLASS 12<sup>th</sup> STUDENTS: A PROSPECTIVE COHORT STUDY

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#### ABSTRACT

**Introduction:** Examinations are the bane of students. Passing or failing usually has strong consequences for one's career development, so examinations cause psychological stress for most of the students.

**Aim:** To assess mental stress on Serum IgA, Gingival status and TMJ disorder among class 12<sup>th</sup> students before and during exam.

**Methodology:** A prospective cohort study was conducted at private tutorial institute, Jaipur. The study population consisted of thirty subjects with age ranging from 16-18 years, who were divided into two groups; group A consisted of Science students and group B Commerce students. Venous blood samples were collected to assess Serum IgA level using immunoassay before and during exams. Clinical evaluation was undertaken using gingival index for assessing Gingival status and TMD prevalence was assessed by using Fonseca's questionnaire before and during exams.

**Results:** In both the groups Serum IgA levels increased during exam and it was more among Science students but results were not statistically significant ( $p \geq 0.05$ ). Gingival inflammation and Mild & Moderate TMD was found to be more among Science students and results were statistically significant. With regards to gender, gingival inflammation was found to be more for males and TMD for females during exams. ( $p \leq 0.05$ )

**Conclusion:** The parallel increase of Serum IgA level, gingival inflammation and TMD during exams in both the groups suggesting a possible relationship between stress and above three parameters.

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## INTRODUCTION

Examinations are the bane of students. However, they have an important role in evaluating students' learning outcomes and their mastery of a subject. Passing or failing usually has strong consequences for one's career development, so examinations cause psychological stress for most of the students (Spangler, 1997). Academic examinations have been considered as one of the most acute stresses experienced by students. (Jemmott and Magloire, 1988; Maes et al., 1997; Maes et al., 1998; Deinzer et al., 2000; Lowe et al., 2000; Lacey et al., 2000) Previous

studies have found that these academic examinations may alter antibody production either behavioral or neurobiological pathways. Academic Stress might alter antibody production through its effect on health practices known to modulate immunity such as smoking, alcohol consumption, sleep and diet. Stress also influences the plasma and tissue concentrations of many hormones that bind to specific receptors on the membrane or in the cytoplasm of cells of the immune system, including the various cells that participate in the production of an antibodies. (Kemeny and Gruenewald, 1999; Rabin, 1999; Lamm, 1997; Ader et al., 2001; Glaser and Kiecolt-Glaser, 1994) Each of the cell types involved in primary and secondary response has receptors for glucocorticoids and catecholamines and binding of these hormones to specific receptors alters

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immune function. Most common antibody which tends to be affected by stress is Serum Immunoglobulins (IgG, IgA and IgM) among which Serum immunoglobulin A (IgA) could be useful diagnostic tool for assessing oral health as it is good indicator of the functional status of the mucosal immune system along with Salivary Immunoglobulin A(IgA) (Glaser and Kiecolt-Glaser, 1994) With chronic stress, the production of immunoglobulin is suppressed, whereas acute psychological challenge increases IgA levels. (Ng *et al.*) Previous studies have utilized Salivary IgA as acute stress situations to study the stress paradigm (Deinzer *et al.*, 2000; Lowe *et al.*, 2000; Lacey *et al.*, 2000). But as India being a developing country, certain tests are not conducted in India and Salivary IgA is one of them. So, instead of Salivary IgA, in current study the parameter used to measure stress was serum IgA as both Salivary IgA and Serum IgA are good indicators of the functional status of immune system in response to physiological stress.

Researchers have indicated that stress is also one of the risk factors for Temporomandibular joint disorder which tends to affect the masticatory system and are able to induce para-functional habits. Temporomandibular disorders is one of the most common disorders in orofacial region that is usually associated with pain, unusual sounds and discomfort in mastication (Hucklebridge *et al.*, 2000; Maes *et al.*, 1997; Greenberg and Glick, 2010) Various prevalence studies have reported that approximately fifty to seventy five percent of subjects exhibit one or more signs of TMD and approximately thirty three percent have at least one symptom. (Rugh *et al.*, 2010; Rollman and Gillespie, 2003; Gray *et al.*, 1994)

Manfredino *et al.* (2009) performed a study using stress measurement questionnaires and indicated that in comparison to other various oral conditions, stress was significantly higher in patients with TMJ disorder ( $p=0.001$ ). Matsuka *et al.* (2008) indicated that stress had an important role in prevalence of TMD and concluded that risk factors such as premature contact, clenching, bruxism and trauma to the joint are of less importance. Since one of the major factor causing stress in students are the academic exams. The epidemiological importance of TMD concerns the knowledge of several symptomatic complexes and therapeutic approaches which allow for the establishment of prevention and control programs. Different questions covering major TMD signs and symptoms have been collaborated to simplify the evolution in epidemiologic studies and to standardize research samples. The anamnestic and clinical indexes proposed by Helkimo's (1974) were obtained from clinical observation. Based on Helkimo's (1974) indexes, Fonseca (1992) developed his anamnestic question that classifies TMD signs and symptoms as mild, moderate or severe or free TMD. (Modi *et al.*, 2012; Stockstill and Callahan, 2007; Deleeuw *et al.*, 2005)

Thus, there are some other factors which have been certainly shown to influence many other parameters of health and disease. Secondly, several studies completed about 10 years ago indicate that there may be strong relationships between stress, depression and poor oral health (Endresen *et al.*, 1987) Most of the epidemiological studies of Serum Immunoglobulin, Oral health and TMD had been done on

western populations thus very sparse data is available for Asian population. Further it has been emphasized that board exam going students are in acute stress and since there has not been any published data regard to assess the effect of stress on these three parameters among board students. Thus, the aim of the present study is to assess the effect of stress on Serum Immunoglobulin IgA, Gingival status and prevalence of Temporomandibular disorder among board students before and during exam.

### Aim

To assess mental stress on Serum IgA, and Gingival status and TMJ disorder among class 12<sup>th</sup> students before and during exams.

### Objectives

1. To assess mental stress on Serum IgA, Gingival status and TMJ disorder among class 12<sup>th</sup> students using Serum Immunoglobulin assay, Gingival index and self-administered questionnaire
2. To assess mental stress and serum immunoglobulin among class 12<sup>th</sup> students using Serum Immunoglobulin Assay.
3. To appraise mental stress and gingival status among class 12<sup>th</sup> students using Gingival index
4. To evaluate mental stress and TMJ disorder among class 12<sup>th</sup> students using a self-administered questionnaire.

## MATERIALS AND METHODS

Ethical approval was obtained from the Ethical Committee of Jaipur Dental College. Written informed consent was obtained from both subjects and their parents (subjects who were <18 years of age) prior to the commencement of the data collection. The clinical examination for every subject was comprehensively carried out by the investigator. In order to assess the intra examiner reliability, the investigator applied the Gingival Index (Loe and Silness, 1963) to record gingival inflammation. The kappa co-efficient value for intra-examiner reliability with respect to the Gingival Index was 0.85. The values reflected high degree of conformity in the observation. TMD prevalence was assessed using self administered questionnaire. The Cronbach alpha value for questionnaire was good (Cronbach alpha value was 0.87) The study was carried out from 10<sup>th</sup> April 2013 to 3<sup>rd</sup> March 2014 in Jaipur city, the capital of Rajasthan and is situated in the north eastern part of the state. The study was carried out in private tutorial institute (PCM Triangle) situated in Sodala, Jaipur. A pilot study was designed and carried out to check the feasibility of the study among a total of 10 students in the age group of 16-18 yrs.' and for the estimation of sample size for the main research in same institute.

### Study population and Sampling Procedure

The study population comprised of 30 school children appearing for 12<sup>th</sup> board exam in the age group ranging between 16-18 yrs.' (16 males and 14 females) which were divided into two Group A and Group B. Each group consisted of 15 students from each Science and Commerce torrent. A

total of 45 subjects in the age group ranging between 16-18 yrs. were screened from the institute and those fulfilling the inclusion criteria were selected for the study. Thus a sample of 30 subjects was obtained who fulfilled all the inclusion criteria of the study.

**Inclusion Criteria:** 12<sup>th</sup> appearing board students

- Subjects having at least 20 natural teeth
- Age - 16-18 years
- Base line scores of gingival index (Loe H modification of Loe H and Silness J, 1963) and plaque index should >0
- Those who agreed to fill the questionnaire.

**Exclusion criteria** -Subjects under any medication affecting immune system

- Tobacco eating/smoking
- Chronic disease affecting immune system
- Malocclusion
- Use of chemical methods of plaque control
- History of professional oral prophylaxis within the last 6 months

**Examination:** Just 10 months before board exam Venous blood samples (3-5 ml) were collected from students between (9-12AM) before taking any medications. Sera were separated and stored at (-20°C) until analysis and same procedure was done during their main exam to assess the correlation between serum IgA level and stress.

**Serum Immunoglobulins A assay:** After placing 5 µl of serum on plates of IgA, levels of both groups were quantitatively studied with immunodiffusion plates (Biomagreb ®). Serum samples were incubated on plates for 72 hours at room temperature. At the end of this period, the diameter of precipitation was measured and converted to mg/dl units using table supplied by the manufacturer. Normal values of the plates used was IgA (60-310mg/dl). Intracomparison and intercomparison was done among both the groups using the student's 't'-test

**TMJ assessment:** The questionnaire proposed by Fonseca (1992) was used to classify TMD severity in the study population because it is highly efficient in obtaining epidemiological data. It is composed of 10 questions, which includes checking for the presence of pain in the temporomandibular joint, head & back, while chewing, parafunctional habits, movement limitations, joint clicking, perception of malocclusion & sensation of emotional stress. The subjects were informed that the 10 questions should be answered with "yes", "no" & "sometimes" & that only one answer should be marked for each question. The Fonseca's questionnaire contains an anamnestic index and the subjects were classified as having mild TMD, moderate TMD, severe TMD or free of TMD (Table 1). The scoring criteria was as follows: A score of '0' indicated the absence of symptoms; a score of '1' was given for a report of an occasional occurrence, a score of '2' was given for each response indicating the presence of dysfunction, and a score of '3' indicated severe pain or bilateral symptoms Positive answers (yes and sometimes) were summed.

## Oral health assessment: Gingival Index by Loe and Silness (1963)

**Follow up:** Examination for Gingival Index (Loe and Silness, 1963), Temporomandibular disorders and serum IgA was carried out at 10 months prior before and during the exam. Subjects were evaluated by the same examiner throughout the study period.

**Table 1. Grading of TMD Degree (Deinzer *et al.*, 2000)**

Score	Categories
0-3	TMD free
4-8	Mild TMD
9-14	Moderate TMD
15-23	Severe TMD

## Analysis

Data thus collected was summarized as Mean and Standard Deviation (Mean±SD). Student t-test was used for analysis of statistical data. For all test a p-value of 0.05 or less was used for statistical significance. Obtained data was compiled systematically and was entered in Statistical Package for the Social Sciences (SPSS) software 19 version.

## RESULTS

The present study was conducted to assess the effect of stress on serum immunoglobulin (IgA), gingival status and TMJ disorder among 12<sup>th</sup> appearing board students. A total of thirty subjects were selected to participate in the study and divided into two groups consisting fifteen subjects in each group.

### Comparison of changes in mean Serum IgA level in both the groups (Table-2)

Student "t" test was applied to assess the difference in the Serum IgA level between the two groups. The test suggested that the change in Serum IgA level between the two groups was statistically insignificant ( $p > 0.05$ ) for the difference between ten months prior and during exam respectively.

### Mean values of Serum IgA before and during main exam regarding gender (Table-3)

The mean values of Serum IgA prior ten months for males and females was 120.05±0.26 and 130±0.56(mg/dl) and during exams was 122.07±0.34 and 137.23±0.23 respectively and the change in mean Serum IgA level was more among females during exams but results were insignificant for both males and females.

### Comparison of changes in mean GI scores in both the Groups (Graph 1)

Student "t" test was applied to know the difference in the gingival index scores between the two groups. The test suggested that the change in gingival scores between the two groups was statistically insignificant ( $p > 0.05$ ) for the difference between ten months prior and during exam respectively.

### Comparison of TMD severity among two groups during exams (Table-5)

When TMJ problems were assessed among both the groups during exams then there was no statistically significant results obtained regarding (Free TMD degree) but for Mild and Moderate TMD degree it had shown statistically significant difference. ( $p < 0.05$ )

### TMD Severity regarding gender before and during exam Table-6,7)

When TMJ problems were assessed then it was observed that females had more prevalence of Mild and Moderate TMD during exams compared to males and results were statistically significant.

Table 2. Serum IgA level among both groups

Groups	Group A	Group B	Unpaired -t-test	p-value
Before (IgA)	135.08±0.53	147.41±0.30	7.23	0.26
During (IgA)	123.01±0.28	130.14±0.14	8.34	0.35

Table 3. Mean values of serum Immunoglobulin A (IgA) levels before and during main exam among Science and Commerce students regarding gender

Gender	Mean Before (IgA)	Mean During (IgA)	Mean diff.	Std.Dv.Diff	Paired -t-value	p-value
Male	120.05±0.26	122.07±0.34	2.02	0.8	7.23	0.33
Female	130.98±0.26	137.23±0.48	6.25	0.22	7.65	0.57

Table-4. Comparison of TMD Severity among two groups during exams

TMD Degree	Group A		Group B		p-value
	N	%	N	%	
Free of TMD	8	53	12	80	0.27
Mild TMD	4	26	2	13	0.04
Moderate TMD	3	21	1	7	0.03

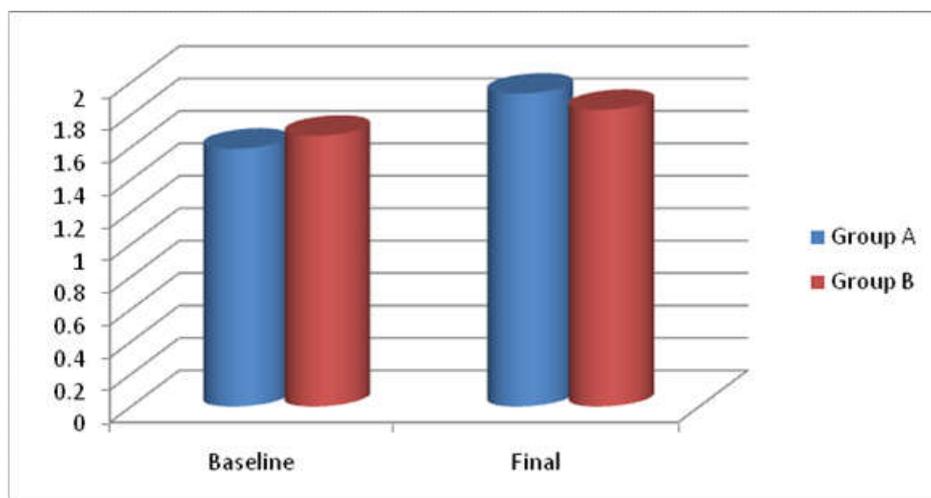
$p < 0.05$

Table 5. Comparison of TMD Severity regarding gender before and during exam

TMD Degree	Male				p-value
	Baseline data		Final data		
	No	Percent	No	Percent	
Free of TMD	12	75	10	62	0.09
Mild TMD	2	12.5	3	19	0.101
Moderate	2	12.5	3	19	0.093

Table 6. Comparison of TMD Severity regarding gender (Female) before and during exam

TMD Degree	Female				p-value
	Baseline data		Final data		
	No	Percent	No	Percent	
Free TMD	12	85	8	57	0.093
Mild TMD	1	7.5	4	29	0.03
Moderate TMD	1	7.5	2	14	0.04



Graph 1. Comparison of GI between the groups before and during the exams

## DISCUSSION

Ronald *et al.* in 1986 conducted study among forty medical students and observed significantly increase in serum immunoglobulins levels (IgG, IgM, and IgA) before and the during examinations, which is accordance with the results of the present study.

Vassend *et al.* in 2006 reported increase in serum immunoglobulin level before, during and after the exam among ten undergraduate students which is par with the results of the present study.

Endresen *et al.* in 1987 conducted study among thirty nurses in emergency ward to assess the effect of their work load on serum immunoglobulin levels and reported significant decrease in serum immunoglobulin levels and results are not concurrent with the results of our study.

A study conducted by Denzier *et al.* in 2001 strongly supported the assumption that psychosocial stress may induce neglect of oral hygiene during their exams and increase of gingival inflammation and showed similar findings with our study. With regard to gender it was found that male students had more gingival inflammation compared to female students which may be explained on fact that in general, female have positive attitude towards health and take better care of their aesthetics than males. also females are more self liking, self competent, usually care more about their corpse and appearance which shows similar results with the study conducted by Ostberg *et al.* 1999; Al-Omari *et al.* 2005; Peker *et al.* in 2009. Aldieris *et al.* in 2010 conducted a study amongst one fifty subjects to assess the effect of psychological stress and TMD and reported that TMD degree had positive relationship with anxiety level which is par with results of the present study.

Mottaghi *et al.* in 2012 conducted a study amongst one thirty university students to assess TMD prevalence before and during the exam and observed that occurrence of TMD were increased during exam which show similar results with the present study. In present study, it was observed that Science torrent students were more in mild and moderate form of TMD compared to Commerce torrent and results were found to be statistically significant. With regards to gender, our result appears concurrent with the findings of Pedroni *et al.* Nomura *et al.* Solberg *et al.* and Ishfaq *et al.* in which they reported high prevalence of sign and symptoms of TMD among women.

## Conclusion

Serum immunoglobulin level (IgA) was found to be more among Science students compared to Commerce students but results were statistically insignificant. Gingival inflammation was found to be more among Science students compared to Commerce students and results were significant. Gingival inflammation was found to be more among males compared to females and results were significant. Mild and Moderate TMD severity was found to be more among Science students compared to commerce students and results were significant.

Mild and Moderate TMD severity was found to be more among females compared to males and results were significant. The parallel increase of serum IgA level, gingival inflammation and TMD during exams in both the groups suggesting a possible relationship between stress and above three parameters. It is recommended further longitudinal studies with larger sample and some other non-invasive procedure with follow up should be carried out to determine the impact of stress/psychological factors on Serum Immunoglobulin A level(IgA), Oral health and TMD prevalence especially students under acute stress.

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