UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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Gustavo Giacomelli Nascimento

O EFEITO DO ESTRESSE PSICOLÓGICO NA IMUNIDADE SALIVAR SECRETÓRIA.

Porto Alegre
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Trabalho de Conclusão de Curso apresentado como requisito para obtenção do grau de Cirurgião-Dentista pela Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul.

Orientador: FERNANDO NEVES HUGO

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“Que pode uma criatura senão,

entre criaturas, amar?”

Carlos Drummond de Andrade

RESUMO

Objetivo: estudar os níveis salivares de Imunoglobulina A Secretória (S-IgA), Imunoglobulina A1 Secretória (S-IgA1), Imunoglobulina A2 Secretória (S-IgA2) e Componente Secretor (SC), assim como as razões S-IgA/SC, S-IgA1/SC e S-IgA2/SC em estudantes universitários, incluindo um grupo apresentou sintomas depressivos, bem como estresse psicológico prolongado.

Materiais e Métodos: a amostra foi constituída por 113 estudantes universitários. Os participantes responderam à Escala de Solidão da UCLA, à Escala de Depressão de Beck (BDI), em sua forma reduzida, e à Escala de Estresse Percebido (PSS). Foi coletada saliva não-estimulada através do método *spitting*. A análise da saliva se deu com o uso do teste ELISA e do teste do ácido bicincôninico (BDA). Para análise estatística foram utilizadas regressões lineares simples e múltiplas.

Resultados: a regressão linear múltipla revelou que o estresse percebido foi negativamente associado com a concentração de IgA1. Os sintomas depressivos foram positivamente associados com a concentração de SC e negativamente associado com as razões IgA1/SC e de IgA2/SC, após ajustes. Solidão foi positivamente associada com a concentração de SC e negativamente associada com as razões IgA1/SC e de S-IgA/SC.

Conclusão: os achados indicam que o estresse psicológico exerceu seus efeitos via células do sistema imunológico, visto que a secreção e o transporte de SC pelas células salivares ductais não foram relacionadas como causadoras da redução da imunidade salivar inata observada.

Palavras-chave: Imunoglobulina A Secretória, Estresse Psicológico, Depressão, Solidão, Saliva.

ABSTRACT

Objective: to study the salivary levels of S-IgA, S-IgA1, S-IgA2 and SC and the S-IgA/SC, S-IgA1/SC and S-IgA2/SC ratios in university students, including a subset of students who were experiencing depressive symptoms and protracted psychological stress.

Methods: 113 undergraduate students took part of this cross-sectional study. The participants answered the UCLA loneliness scale, the Beck Depression Inventory [short form] and the Perceived Stress Scale. Unstimulated saliva was collected following the *"spitting method"*. Saliva was analyzed using ELISA tests and the bicinchoninic acid method. Statistical analyses were accomplished using univariate and multiple linear regressions.

Results: Multiple linear regression analyses revealed that perceived stress was negatively associated with IgA1 concentration. Depressive symptoms were positively associated with SC concentration and negatively associated with IgA1/SC ratio, IgA2/SC ratio after adjustment for confounders. Loneliness was positively associated with SC concentration, and negatively associated with IgA1/SC ratio and S-IgA/SC ratio.

Conclusion: These findings indicate that psychological distress exerts its effects through immune cells, since the output and transportation of SC by salivary duct cells were not attributable as the causes of stress-related innate immunity impairment observed in the present study.

Key words: Secretory immunoglobulin A, Psychological stress, Depression, Loneliness, Saliva.

SUMÁRIO

1. INTRODUÇÃO

Existem evidências que demonstram a relação existente entre o estresse psicológico crônico, demonstrado através de episódios estressores, e doenças e infecções do trato respiratório (COHEN *et al.*, 1991, 1993). Mais de 95% de todas as infecções se iniciam na superfície mucosa (BOSCH *et al.*, 2002). Essas superfícies são protegidas por várias proteínas originadas de glândulas exócrinas, entre elas as glândulas salivares. A maior parte dessas proteínas constitui-se de imunoglobulinas. Logo, o efeito do estresse psicológico na suscetibilidade a infecções, especialmente as do trato respiratório, pode ser explicada por essa queda na concentração salivar de imunoglobulinas. A liberação glandular dessas proteínas protetoras é controlada fortemente pelo sistema nervoso central autônomo (GARRET, KIDD, 1976). É sabido que o stress psicológico regula o funcionamento desse sistema, e que os fatores estressantes acabam influenciando o sistema imune (BOSCH *et al.*, 2002).

O marcador característico da imunidade mucosa é a imunoglobulina A secretora (S-IgA). S-IgA é a primeira imunoglobulina dentre as secretadas pela mucosa, e a principal a ser usada em pesquisas onde se avaliam os efeitos do estresse psicológico sobre a imunidade secretora (NORDERHAUG *et al.*, 1999, BOSCH *et al.*, 2002). Embora algumas evidências sugiram que o estresse está associado a uma diminuição nos níveis de S-IgA, a literatura apresenta achados inconsistentes. Uma revisão sistemática identificou duas possíveis causas para essa inconsistência (BOSCH *et al.*, 2001). Primeiramente, a maioria dos estudos tem usado pequenas amostras populacionais, o que impossibilita detectar associações estatisticamente significativas. Em segundo lugar, alguns estudos mensuraram o estresse psicológico usando questionários de qualidade psicométrica desconhecida, diminuindo, então, a probabilidade de associações estatísticas.

Diversas outras limitações têm sido encontradas na literatura (BOSCH *et al.*, 2001). Entre elas, o fato de os estudos usarem a concentração total de S-IgA, ignorando a existência subclasses da S-IgA, IgA1 e IgA2, que são encontradas na saliva em uma taxa de 3:2 (BRANDTZAEG *et al.*, 1999). A diferenciação entre essas classes é de suma importância, uma vez que a diminuição dos níveis de IgA1, mas não de IgA2, está associada a um aumento no risco de infecções no trato respiratório superior (GLEESON *et al.*, 1999). Interessantemente, tanto o aumento do estresse

agudo quanto um exercício físico de curta duração acabam diminuindo os níveis de IgA1, o que não acontece com o IgA2 (BOSCH *et al.*, 2001). Isso sugere que a secreção dessas duas subclasses se dá por mecanismos de controle diferentes, e podem, portanto, serem diferentemente afetadas pelas distintas formas de estresse psicológico.

Uma segunda limitação encontrada na literatura é que não se sabe se os efeitos do estresse sobre S-IgA são causados por uma diminuição da liberação de IgA pelas células B, ou por uma diminuição do transporte do IgA pelas glândulas salivares até que seja constituída a saliva. Os linfócitos B produtores de IgA estão presentes localmente no tecido glandular salivar. Essas células B produzem uma forma polimérica do IgA (pIgA), que quando secretado na saliva assume a forma de S-IgA (NORDERHAUG *et al.*, 1999). Um transportador molecular intermedia a secreção, denominado de Componente Secretor (SC), que une e desloca pIgA do interior do tecido glandular até a saliva (o complexo SC-pIgA compõe a molécula de S-IgA). O SC também pode se deslocar sem ter consigo o pIgA, quando somente o SC é secretado com a saliva. Consequentemente, o S-IgA salivar e o SC são partes componentes do sistema S-IgA independentemente secretadas, e são indicadores da atividade transportadora das células B e do tecido glandular respectivamente. Essencialmente, a mensuração de ambas as S-IgA e a taxa entre S-IgA e SC ajudarão a responder se os efeitos do estresse nos níveis de S-IgA são devidos a uma diminuição da viabilidade da imunoglobulina (ou seja, queda na liberação pelos linfócitos B), ou devidos a uma deficiência das glândulas em transportar essa imunoglobulina até a mucosa.

2. ARTIGO PARA PUBLICAÇÃO

THE EFFECTS OF PSYCHOLOGICAL DISTRESS ON SALIVARY SECRETORY IMMUNITY.

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ABSTRACT

Objective: to study the salivary levels of S-IgA, S-IgA1, S-IgA2 and SC and the S-IgA/SC, S-IgA1/SC and S-IgA2/SC ratios in university students, including a subset of students who were experiencing depressive symptoms and protracted psychological stress.

Methods: 113 undergraduate students took part of this cross-sectional study. The participants answered the UCLA loneliness scale, the Beck Depression Inventory [short form] and the Perceived Stress Scale. Unstimulated saliva was collected following the *"spitting method"*. Saliva was analyzed using ELISA tests and the bicinchoninic acid method. Statistical analyses were accomplished using univariate and multiple linear regressions.

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Conclusion: These findings indicate that psychological distress exerts its effects through immune cells, since the output and transportation of SC by salivary duct cells were not attributable as the causes of stress-related innate immunity impairment observed in the present study.

Key words: Secretory immunoglobulin A, Psychological stress, Depression, Loneliness, Saliva.

INTRODUCTION

Human and animal studies have provided convincing evidence that link psychological stress to increased susceptibility to infectious diseases and poorer health outcomes (COHEN, 1996, ENGELAND & MARUCHA, 2009). Approximately 95% of all infections start at mucosal surfaces (BOSCH, 2002). The mucosa is protected by the mucous secretions of various exocrine glands, such as the salivary glands (AMERONGEN 1995). Anti-microbial proteins (e.g, immunoglobulins) present in these secretions constitute a first line of immunological defenses that prevent infection and disease through interference with, and regulation of microbial colonization (RUDNEY 1995; SCHENKELS, 1995). Glandular release of these protective proteins is under strong autonomic nervous system control (GARRET, 1976). As psychological stress is known to modulate autonomic nervous system functioning (BERNTSON, 1993, SANDERS AND STRAUB, 2002, ENGELAND AND MARUCHA, 2009), psychological stressors are likely to exert an influence on this aspect of immunity (BOSCH *et al.*, 2002).

The hallmark of mucosal immunity is secretory immunoglobulin A (S-IgA). S-IgA is the primary immunoglobulin in mucosal secretions (NORDERHAUG, 1999, FAGARASAN & HONJO, 2003) and the main outcome measure used in research on the effects of stress on secretory immunity (BOSCH *et al.*, 2002). Although the balance of evidence indicates that protracted stress is associated with decreased levels of S-IgA, the literature yields inconsistent findings. A systematic review identified two possible causes for this inconsistency (BOSCH *et al.*, 2001). First, most studies have used small sample sizes that were likely insufficient to detect statistically significant associations. Second, a number of studies assessed psychosocial stress using questionnaires of unknown or sub-optimal psychometric quality, further reducing the probability of significant associations.

Several other limitations in the literature have been noted as well (BOSCH *et al.*, 2001). Nearly all studies have only utilized total S-IgA, ignoring the existence of distinct IgA subclasses. These subclasses, denoted IgA1 and IgA2, are found in saliva in a ratio of 3:2 (BRANDTZAEG, 1999). Differentiating between these subclasses may be relevant because decreased salivary IgA1 levels, but not IgA2 levels, are associated with an increased risk of upper respiratory tract infections (GLEESON 1999, COHEN *et al.*, 1991, 1993). Interestingly, both

acute psychological stress and brief exercise decrease the concentrations of IgA1, but not IgA2 (BOSCH, 2001). This suggests that the secretion of the two subclasses is under differential control, and may therefore also be differentially affected by protracted forms of psychological stress.

A second limitation in the literature is that it is unknown if the effects of stress on S-IgA are caused by reduced release of IgA by B cells or by reduced transport of IgA into saliva by the salivary glands. The IgA-producing B lymphocytes are present locally in salivary gland tissue. These B cells produce a polymeric form of IgA (pIgA) that is ultimately secreted into saliva as S-IgA (NORDERHAUG, 1999, SUN *et al.*, 2004). A transporter molecule mediates this secretion, denoted as Secretory Component (SC), which binds pIgA and translocates it from the inside of the glandular tissue into saliva (the SC-pIgA complex composes the S-IgA molecule). SC can also translocate without pIgA being attached to it, in which case only SC is secreted into saliva. Thus, salivary S-IgA and SC are partly independently secreted components of the S-IgA system that are indicators of B-cell and glandular transport activity respectively. Essentially, measuring both S-IgA and the ratio between S-IgA and SC will help determine if the effects of stress on S-IgA levels are due to a decreased availability of immunoglobulin (i.e., decreased released by the B lymphocytes) or due to a decrease in the ability of the glands to transport this immunoglobulin into saliva.

In the light of the preceding discussion the aim of the current investigation is to study the salivary levels of S-IgA, S-IgA1, S-IgA2 and SC and the S-IgA/SC, S-IgA1/SC and S-IgA2/SC ratios. This was done in university students, including a subset of students who were experiencing depressive symptoms and protracted psychological stress. Specifically, these individuals were subdivided based on lower or higher scores on depression and loneliness scales.

METHODS

Participants

Undergraduate volunteers (N=113, 61 male, mean age 20.7 (±3.0), range 17-33) were recruited from the student population at The Ohio State University in Columbus, Ohio between November 2002 and August 2003. The study protocol was reviewed and approved by the Institutional Review Board (IRB: 2008-0124). Recruitment took place during organized meetings in dormitories and classrooms, and through local advertisements. Exclusion criteria included the use of medications or health problems (e.g., inflammatory, endocrine) that have known effects on immune or salivary gland function, oral health problems that require prompt treatment, and infections within the 2 weeks before assessment. Participants provided informed consent and received financial compensation for their time and effort. This present study was based on a sub-sample of a larger study described elsewhere (BOSCH *et al.*, 2007).

Procedures

Participants were scheduled between 10:00 am and 11:00 am to minimize the influence of circadian variation. In preparation for the study, participants were instructed not to engage in strenuous physical exercise, and to refrain from using alcohol or non-prescription drugs 24 hours before the experimental sessions. In addition, participants were instructed to abstain from caffeine the day of the experiment, to eat breakfast before 9:30 a.m., and to eat or drink nothing after that point (except for water).

Upon arrival at the School of Dentistry Research Clinic of The Ohio State University, participants filled out questionnaires to assess psychological traits and states, demographics, and health behaviors. After 30 minutes the saliva collection started.

Questionnaires

Demographic and health behavior measures

Questionnaires were used to assess a number of variables that might confound or mediate the relation between psychosocial factors and saliva secretory immunity. These included demographic factors such as gender, age, and ethnicity, as well as health practices and life style variables such as alcohol, caffeine and tobacco consumption.

Psychological measures

The Beck Depression Inventory (BDI) Short Form is a widely used and well validated self-report measure of depressive symptoms that boasts good reliability statistics, correlates well with other measures of depression, and predicts clinical ratings of depression. In the present study, internal reliability was high (Cronbach's α=0.84). The scale comprises 13 items assessing the severity of depressive symptoms experienced during the past two weeks, on a four-point Likert scale, with values ranging from 0 to 3.

Loneliness, the negative emotional experience of being socially isolated, was measured using the Revised UCLA Loneliness Scale (UCLA-R). For this 20-item scale participants again responded using a four-point Likert scale format, with answers ranging from often to never. Scores correlate with measures of shyness and self-esteem, and high scores on this measure have been associated with alterations in immune system functioning (HAWKLEY AND CACIOPPO, 2003; UCHINO *et al.*, 1996). This scale is acknowledged to have good psychometric properties (RUSSELL *et al.*, 1980). In this study, internal reliability was again high (Cronbach's α=0.94).

The Perceived Stress Scale (PSS) is a 10-item scale that assesses the degree in which situations in one's life are appraised as taxing and stressful. The questions assess thoughts and feelings about stressful events, control, overload and experienced stress, as well as how often the individual felt or thought in a stressful manner. The scale allows for the examination of stress pathology relationships and has been found to predict mental and physical health outcomes (COHEN *et al.*, 1993). In this study, internal reliability for the PSS was high (Cronbach's $\propto$=0.85).

Saliva collection

Before collection participants were provided with water (ad lib.) to avoid dehydration until 10 minutes before the start of the collection trial. At that point participants were requested to rinse the mouth with water. Unstimulated saliva was collected by the "spitting method" according to the directions given by Navazesh (1993). The collection trial started with the instruction to void the mouth of saliva by swallowing. Subsequently, saliva was allowed to accumulate in the floor of the mouth, without stimulation of secretion by means of oro-facial movements. The participant spat into an ice-chilled polypropylene test tube every 60 seconds. Unstimulated saliva was collected for 10 minutes. After collection saliva was homogenized using a vortex mixer and centrifuged (10,000 x g, 6 min, 4°C) to eliminate buccal cells and oral microorganisms. The clear supernatant was divided into small aliquots and stored at -80°C until use.

Assessment of S-IgA, IgA subclasses, and Secretory Component

The sandwich ELISAs for total S-IgA, S-IgA1, S-IgA2 and SC were performed in duplicate as described by Bosch *et al.* (Bosch *et al.*, 2001) with small modifications. All assays used a capture antibody that was directed against the Secretory Component (Clone GA-1, Sigma-Aldrich, Saint Louis, MO, USA). The high specificity and quality of this monoclonal antibody has been established in a multicentre WHO/NIUIS study (MESTECKY *et al.*, 1996) and corroborated in our laboratory by Western blot and ELISA. For detection, we used HRP-conjugated mouse anti-human monoclonal antibodies directed against S-IgA, IgA1, IgA2, or SC (all obtained from Nordic Immunology, Tilburg, Netherlands). These monoclonal antibodies have been found of high specificity in several comparative studies (DE FIJTER *et al.*, 1995; MESTECKY *et al.*, 1996; REIMER *et al.*, 1989). We found that these ELISAs did not give a positive signal when secretions (saliva, breast milk) of IgA-deficient individuals or normal serum were tested, which further supports that test values were not affected by other secretory fluid or serum components. The standards for S-IgA and SC were also obtained from Nordic (IgA1, IgA2). The intra-assay variability of the assays were <4% (inter assay CV% < 6%). The ELISAs

were all found to be of very high sensitivity, with upper detection limits (> 200pg/ml) that were several hundred-fold above the lowest observed concentrations.

Determination of total salivary protein concentrations

Total protein was determined using the bicinchoninic acid method (Bicinchoninic acid solution, Sigma, Saint Louis, MO, USA; Copper (II) sulfate solution, Sigma-Aldrich, Saint Louis, MO, USA), as described by Bosch *et al.* (1996). The intra-assay variability of each assay was<5% (inter assay CV% <10%).

Statistical analyses

Linear regression analyses were performed to assess relationships among psychosocial factors and salivary parameters with the following outcomes: S-IgA, IgA1, IgA2, SC, SIgA/SC ratio, IgA1/SC ratio and IgA2/SC ratio. Initially, simple linear regression was used to establish whether there were associations between psychosocial factors and salivary levels of SIgA, IgA1, IgA2 and SC. In subsequent hierarchical regression analysis, age and gender (demographics variables) were entered at step 1. After that, an adjustment for smoking, alcohol and caffeine consumption (health behaviors) were performed in step 2. The importance of controlling for smoking behavior when examining possible associations between mucosal immunity and psychosocial variables has been demonstrated (EVANS, 2000). For the step 3, to account for dilution effects of secretory immunoglobulin and SC levels, values were corrected for salivary protein concentrations. The same analyses were also performed IgA1/SC , IgA2/SC ratio and SIgA/SC ratio outcomes.

Altered levels of these salivary factors could be due to changes in IgA production by plasma cells or S-IgA transport by glandular cells. Importantly, reduced availability of S-IgA relative to the ability to transport this immunoglobulin would be reflected in a decreased S-IgA/SC ratio.

Because salivary flow rate (ml/min) was not recorded, total protein was used as a covariate for the analyses on S-IgA/SC, IgA1/SC, IgA2/SC ratios (NYKLICEK *et al.*, 2005). The rationale behind this approach is that dilution effects due to variation in flow rate will similarly

affect total protein concentration and S-IgA concentration. Hence, variance in S-IgA concentration that is secondary to variation in flow rate will be removed by controlling for total protein concentration.

RESULTS

Approximately half of the participants were male (N=61, 54%) and most were Caucasian (N=86, 76%). The mean age of the study sample was 20.7 years. Values S-IgA, IgA1, IgA2 or SC that were >3 S.D. from the mean (<2%) were considered as outliers, and removed from the analyses.

Univariate linear regression analyses revealed that S-IgA was associated with salivary protein concentration (β=0.121, t=3,932, R^2=0,125, p<0.05) and IgA2 with gender (β =-29.275, t=-1,684, R^2=0.025, p<0.05), age (β=7,997, t=2,875*, R^2=0,069, p<0.05) and salivary protein concentration (β=0,067, t=2,235, R^2=0,044, p<0.05). In addition, IgA1 was associated with salivary protein concentration (β=0,096, t=3,972, R^2=0,127, p<0.05) and with perceived stress (β=-1,742, t=-1,454, R^2=0,063, p<0.01). For SC there were significant associations with salivary protein concentration (β=0,485, t=2,103, R^2=0,039, p<0.05), depressive symptoms (β=69,716, t=2,955, R^2=0,084, p<0.01) and loneliness (β=17,081, t=2,485, R^2=0,059, p<0.05). There was a statistically significant association between IgA1/SC ratio and gender (β=-0,052, t=-2,083, R^2=0,038, p<0.05), depression (β=-0,011, t=-2,467, R^2=0,060, p<0.05), perceived stress (β=-0,004, t=-2,516, R^2=0,061, p<0.05) and loneliness (β=-0,011, t=-2,467, R^2=0,060, p<0.05). IgA2/SC ratio was significantly associated with age (β=0,013, t=2,884, R^2=0,07, p<0.01) and depressive symptoms (β=-0,010, t=-2,076, R^2=0,043, p<0.05).

Multiple linear regression analyses revealed that perceived stress was negatively associated with IgA1 concentration (ß=-2.09, t=-2.29, R^2 change=0.04, p<0.05), and IgA1/SC ratio (ß=-0.003, t=-2.10, R^2 change=0.04, p<0.05) after adjustment for age and gender (step 1), smoking, alcohol and caffeine intake (step 2), and total saliva protein concentration (step 3) (Tables 1 and 2). Depressive symptoms were positively associated with SC concentration (ß=61.88, t=2.61, R^2 change=0.06, p<0.05), and negatively associated with IgA1/SC ratio (ß=-0.01, t=-2.42, R^2 change=0.05, p<0.05), IgA2/SC ratio (ß=-0.01, t=-2.05, R^2 change=0.04, p<0.05) after adjustment for confounders (Table 3 and 4). Loneliness was positively associated with SC concentration (ß=17.67, t=2.65, R^2 change=0.06, p<0.01), and negatively associated with IgA1/SC ratio (ß=-0.004, t=-2.87, R^2 change=0.07, p<0.05) and S-IgA/SC ratio (ß=-0.003, t=-2.69, R^2 change=0.06, p<0.05), also after adjustment for confounders (Tables 5 and 6).

DISCUSSION

The present study provided an extensive analysis of the associations between secretory immunity (salivary S-IgA, S-IgA subclasses, Antibody/SC ratios) and measures of psychosocial distress (depressive symptoms, psychological stress, and loneliness) in a sample of healthy young adults. To our knowledge, this is the first study confirming that psychological distress exerts differential effects in different salivary IgA subclasses output. The results showed that S-IgA concentrations and the antibody/SC ratios were both significantly associated with the distress measures. Generally speaking, higher perceived stress, depression symptoms and loneliness scores resulted in lower S-IgA levels, in particular S-IgA1, and lower antibody/SC ratios. Such results support the influence of psychological distress in the production of S-IgA and its subclasses by B Lymphocites (GALLAGHER *et al.*, 2008). In the other hand, SC concentrations were positively affected by psychological distress (e.g. increased distress resulted in increased SC). These findings indicate that psychological distress exerts its effects through immune cells, since the output and transportation of SC by salivary duct cells were not attributable as the causes of stress-related innate immunity impairment observed in the present study.

By statistical adjustment for salivary protein concentrations we were able to establish that the observed relationships between psychosocial measures and secretory immunity were not driven by variations in salivary flow rate. Although the associations reported in the present study were mostly in the predicted direction, the observed associations were fairly modest in size.

S-IgA and IgA1 concentrations and ratios were negatively associated with perceived stress, depression symptoms and loneliness, while IgA2 concentrations and ratios were negatively associated only with depression symptoms, indicating the effects of distress on salivary immunity may be selective. This finding is consistent with Bosch *et al.* (2001) who also found that S-IgA2 levels were not affected by acute stressors which did, however, affect the levels of total S-IgA and S-IgA1. The decrease in salivary S-IgA due to psychological distress was probably due to a decrease in the synthesis of antibodies by local plasma cells, which is opposite of previous evidence examining the effects of acute stress in innate immunity (BOSCH *et al.*, 2002).

The data for acute stressors support an upregulation of natural immunity, the exception is the increased secretion of IgA antibody, which is a product of the specfic immune response; 1) CD8 cells are also upregulated, and these cells also belong to adaptive immunity 2) S-IgA may be in part innate. An interesting question for future research is whether this effect is part of a larger non-specific protein release in the oral cavity in response to acute stress (BOSCH *et al.*, 2002).

S-IgA is a measure of adaptive immune response, that is responses that are driven by exposure to a previously encountered antigen. If this was indeed the case, then only the levels of S-IgA molecules that are specially directed against particular antigens would matter for host protection rendering the measurement of total S-IgA levels less meaningful. However, total S-IgA consists, for the most part, of so-called 'natural antibodies' (BOSH *et al.*, 2000). These natural antibodies have a broader speficity to recognize many different types of antigen, and their levels are independent of reexposure to specific antigens (STOEL *et al.*, 2005). Hence, total immunoglobulin levels are the key to monitoring this aspect of immunity. This perspective is consistent with the evidence showing that total S-IgA levels are predictive of susceptibility to respiratory, oral, and aural infections (JEMMOTT AND MCCLELLAND, 1989). Thus, total S-IgA levels is an immunologically meaningful measure of mucosal host resistance.

Some limitations of this study must be taken into consideration. The sample consisted of young university students and the generalization of our findings to other age and socio-demographic groups needs to be established. Interestingly Phillips *et al.* similarly found a negative correlation between life stress symptoms and salivary S-IgA in a large epidemiological study that consisted of several age cohorts with a broad social-economic variety (PHILLPS *et al.*, 2006). It also remains to be demonstrated to what extent our findings generalize to other models of distress like work stress or psychiatric disorders. Finally, secretory immunity, measured as S-IgA/SC, IgA1/SC and IgA2/SC ratios, controlled for salivary flow rate by correcting for changes in total protein concentrations. One drawback of this approach is that protein secretion and secretory immunity secretion may share variance other than caused by changes in flow rate. This might result in an attenuation of the observed effects (NYKLICEK *et al.*, 2005).

In sum, the findings of the present study show that depressive symptoms, loneliness and psychological stress were negatively associated with down-regulation of various aspects of

secretory immunity. A novel finding was that in our study we could see the influences themselves separately acting in different subclasses of S-IgA, and how they change according to those feelings. Besides, we can look at mechanism of S-IgA production and identify the immune system that is involved. These associations did not appear to be driven by variations in health behaviors or demographic characteristics, or saliva secretion. Given the importance of S-IgA in immune defense at mucosal surfaces and the frequency with which infections are initiated at these surfaces, S-IgA dysregulation could be a means by which psychological stress increases susceptibility to infectious diseases.

TABLES

Table 1. Beta values and R^2 change of multiple linear regression using perceived stress symptoms (PSS) to predict S-IgA, IgA1, IgA2 and SC.

		S-IgA			IgA1			IgA2			SC		
		β	t	R^2 Change	β	T	R^2 Change	β	t	R^2 Change	β	t	R^2 Change
Step 1	Gender	-23.78	-1.70	0.04	-17.83	-1.23	0.04	-14.44	-0.82	0.09**	38.62	0.27	0.006
	Age	-6.18	-2.09*		1.47	0.63		7.79	2.76**		-15.70	-0.69	
Step 2	Smoking	-22.16	-1.00	0.007	-12.52	-0.72	0.005	18.60	0.88	0.03	62.36	0.37	0.005
	Alcohol	3.81	0.19		-0.40	-0.03		21.58	1.13		-11.88	-0.08	
	Caffeine	-0.82	-0.04		7.05	0.46		-21.18	-1.14		2.48	0.02	
Step 3	Protein	0.13	4.14**	0.13**	0.09	3.80**	0.11**	0.05	1.73	0.02	0.52	2.19*	0.05*
Step 4	PSS	-2.15	-1.85	0.03	-2.09	-2.29*	0.04*	-1.20	-1.08	0.01	12.64	1.41	0.02

*p<0.01
**p<0.05

Table 2. Beta values and R^2 change of multiple linear regression using perceived stress (PSS) to predict IgA1/SC, IgA2/SC and S-IgA/SC .

		IgA1/SC			IgA2/SC			S-IgA/SC		
		β	t	R^2 Change	β	T	R^2 Change	β	t	R^2 Change
Step 1	Gender	-0.04	-1.45	0.06*	0.03	-1.18	0.09**	-0.05	-1.54	0.03
	Age	0.005	1.28		0.01	2.88**		0.00	-0.17	
Step 2	Smoking	-0.03	-0.97	0.01	0.01	0.23	0.01	-0.04	-1.05	0.01
	Alcohol	0.01	0.44		0.03	0.85		0.008	0.22	
	Caffeine	-0.01	0.40		-0.03	-0.85		-0.01	-0.40	
Step 3	Protein	-0.00	0.25	0.01	-0.00	-0.59	0.003	-0.00	0.84	0.007
Step 4	PSS	-0.003	-2.10*	0.04*	-0.001	-0.67	0.004	-0.003	-1.61	0.02

*p<0.05
**p<0.01

Table 3. Beta values and R^2 change of multiple linear regression using depressive symptoms (BDI) to predict S-IgA, IgA1, IgA2 and SC.

		S-IgA			IgA1			IgA2			SC		
		β	t	R^2 Change	β	T	R^2 Change	β	t	R^2 Change	β	t	R^2 Change
Step 1	Gender	-30.12	-1.64	0.04	-22.87	-1.58	0.04	-15.10	-0.88	0.09**	35.89	0.26	0.006
	Age	-5.78	-1.93		1.875	0.74		7.73	2.75**		-14.01	-0.63	
Step 2	Smoking	-22.28	-0.99	0.007	-11.39	-0.64	0.005	21.72	1.03	0.03	17.54	0.11	0.005
	Alcohol	1.40	0.07		1.18	-0.07		24.24	1.27		-56.31	-0.35	
	Caffeine	-1.48	-0.08		7.23	0.46		-19.46	-1.05		-21.62	-0.15	
Step 3	Protein	0.13	4.16**	0.14**	0.10	3.97**	0.12**	0.06	2.05*	0.03	0.38	1.82	0.04*
Step 4	BDI	-1.34	-0.42	0.01	-2.82	-1.13	0.01	-4.62	-1.55	0.02	61.88	2.61*	0.06*

*p<0.05
**p<0.01

Table 4. Beta values and R^2 change of multiple linear regression using depressive symptoms (BDI) to predict IgA1/SC, IgA2/SC and S-IgA/SC .

		IgA1/SC			IgA2/SC			S-IgA/SC		
		β	t	R^2 Change	β	T	R^2 Change	β	T	R^2 Change
Step 1	Gender	-0.04	-1.64	0.06*	-0.03	-1.11	0.09**	-0.06	-1.69	0.03
	Age	-0.05	1.30		0.01	2.83**		0.00	-0.17	
Step 2	Smoking	-0.02	-0.75	0.01	0.02	0.45	0.01	-0.04	-0.89	0.01
	Alcohol	0.02	0.61		0.03	1.11		0.01	0.35	
	Caffeine	-0.01	-0.27		-0.02	-0.71		-0.01	-0.31	
Step 3	Protein	0.00	0.80	0.001	-0.00	-0.16	0.003	0.00	1.25	0.007
Step 4	BDI	-0.01	-2.42*	0.05*	-0.01	-2.05*	0.04*	-0.01	-1.83	0.03

*p<0.05
**p<0.01

Table 5. Beta values and R^2 change (between brackets) of multiple linear regression using loneliness symptoms (UCLA) to predict S-IgA, IgA1, IgA2 and SC.

		S-IgA			IgA1			IgA2			SC		
		β	t	R^2 Change	β	T	R^2 Change	β	t	R^2 Change	β	T	R^2 Change
Step 1	Gender	-32.74	-1.76	0.04	-29.05	-2.02*	0.04	-18.84	-1.07	0.09**	148.44	1.07	0.006
	Age	-5.89	-1.95		1.39	0.59		8.03	2.81**		-9.91	-0.44	
Step 2	Smoking	-24.00	-1.07	0.007	-15.37	-0.88	0.005	17.79	0.84	0.03	94.66	0.57	0.005
	Alcohol	-0.35	-0.02		-5.05	-0.32		19.38	1.02		24.95	0.17	
	Caffeine	-0.77	0.04		9.57	0.61		-21.66	-1.15		-56.96	-0.32	
Step 3	Protein	0.13	4.18**	0.14**	0.10	3.91**	0.12**	0.05	1.76	0.03	0.48	2.07*	0.04*
Step 4	UCLA	-0.43	-0.48	0.002	-1.16	-1.66	0.02	-0.09	-0.10	0.00	17.67	2.65**	0.06**

*p<0.05
**p<0.01

Table 6. Beta values and R^2 change of multiple linear regression using loneliness symptoms (UCLA) to predict IgA1/SC, IgA2/SC and S-IgA/SC .

		IgA1/SC			IgA2/SC			S-IgA/SC		
		β	t	R^2 Change	β	T	R^2 Change	β	t	R^2 Change
Step 1	Gender	-0.06	-2.43*	0.06*	-0.05	-1.60	0.09**	-0.07	-2.22*	0.03
	Age	0.005	1.09		0.01	2.74**		-0.002	-0.28	
Step 2	Smoking	-0.04	-1.20	0.01	0.004	0.13	0.01	-0.05	-1.20	0.01
	Alcohol	0.003	0.13		0.02	0.74		0.00	-0.01	
	Caffeine	-0.002	-0.08		-0.02	-0.66		-0.001	-0.20	
Step 3	Protein	0.00	0.44	0.001	-0.00	-0.51	0.003	-0.00	0.98	0.007
Step 4	UCLA	-0.003	-2.69**	0.06*	-0.002	-1.41	0.02	-0.003	-1.77	0.03

*p<0.05
**p<0.01

3. CONSIDERAÇÕES FINAIS

Com base nos resultados apresentados, novos estudos abordando a importância de desconforto psicológico na imunidade salivar mediada por imunoglobulinas salivares em populações distintas, como adultos e idosos são fundamentais para uma melhor compreensão desses efeitos, especialmente no que se refere ao seu mecanismo, seja esse relacionado com a síntese das imunoglobulinas pelas células imunes, seja pelo transporte via células ductais das glândulas salivares. Além disso, o uso de amostras com maior idade permitirá que se possa observar com mais clareza as possíveis influências da idade avançada juntamente com os fatores estressores na função imune salivar.

Os resultados encontrados nos levam a crer que o estresse psicológico está diretamente ligado à produção da S-IgA, influenciando o processo de formação de tais moléculas pelos linfócitos B, não demonstrando, portanto, relação com o transporte através do interstício glandular, pois os níveis de Componente Secretório foram mantidos, mesmo com a queda na produção da S-IgA. Além disso, o estresse psicológico parece ter mais efeito sobre o IgA1, a subdivisão da S-IgA que está diretamente ligada à proteção da mucosa em casos de infecção.

Assim, as evidências apresentadas parecem suportar que o estresse psicológico exerce nítido efeito sobre a produção da S-IgA, ou seja, acaba por diminuir a imunidade da mucosa na região de trato respiratório superior, tornando-a mais susceptível a infecções. Estudos de intervenção examinando o efeito de terapia e/ou suporte psicológico na imunidade salivar e na prevenção de infecções de vias aéreas superiores em grupos de pessoas vivenciando desconforto psicológico podem trazer novos e interessantes achados sobre os mecanismos envolvidos na alteração da imunidade mediada por estresse psicológico, bem como resultar em melhor qualidade de vida e menos morbidade por infecção na população como um todo.

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