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Review Article

Comparison of salivary immunoglobulin A and cortisol levels in competitive and non-competitive child male swimmers

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Abstract

Background: Competitive swimming leads to changes in salivary immunoglobulin A (S-IgA) and cortisol levels. The intensity and duration of exercise cause changes in the immune system. S-IgA and cortisol play a key role in maintaining the body's immunity. This study was performed to investigate the effect of swimming exercise on the immune and hormonal systems of male children with the same caries index in Zanjan, Iran.

Methods: In this cross-sectional study, 86 boys 6–12 years old were selected. Based on their duration of training, the children were divided into two groups of 43: The competitive group and the non-competitive group. The group matching method in terms of dental caries, anxiety, and age was performed in the two groups. Stimulated saliva samples were collected to measure S-IgA and cortisol levels by ELISA before and after the swimming test. Independent *t* test, paired *t* test, and chi-square test were used for statistical analysis of data, with the significance level set at $P<0.05$.

Results: The amount of S-IgA was higher in the competitive than in the non-competitive group. After swimming training, S-IgA increased in both age groups (28 children aged 6–9 and 58 aged 9–12 years old). In the 6–9 age group, the amount of cortisol in non-competitive boys increased significantly ($P=0.048$). In the 9–12 age group, the amount of cortisol in the competitive boys decreased.

Conclusion: Based on the findings of this study it seems that swimming can increase the amount of cortisol in the 6-9 age group. However, short-term physical activity (swimming test in this study) could not make a significant difference in the immune and hormonal systems of either of the age groups. Further studies are suggested to provide information on changes in the caries index.

Keywords: Exercise, Immunoglobulin A, Saliva, Cortisol, Dental caries, Child, Boys

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Introduction

As an immunological agent, S-IgA plays an important role in controlling dental caries by preventing the attachment of caries-causing microorganisms to hard tooth surfaces and inhibiting the activity of the glycotransferase enzyme, as well as maintaining stable environmental conditions with the help of salivary cortisol.[1–3].In general, dental problems have a significant impact on a person's quality of life and self-confidence.[4] Sugar yeast microorganisms and changes in the immune system and hormones are effective factors in the formation of caries.[5] Microorganisms interact with the immune system in multiple ways, and the defense of the host does not go unchallenged.[6] Due to the insufficient development of cellular immunity in children, the barrier created by humoral immunity is very important in preventing infections.[7] Salivary immunoglobulin A (S-IgA) acts as the first line of defense against pathogens.[8] This immunological agent prevents caries by inhibiting the attachment of microorganisms to the tooth surface.[2,9] It provides this by preventing the activation of the enzyme glycosyltransferase in pathogens.[2,10] Exercise and stress have been reported to be associated with caries.[2,4,8,9] As a biomarker of stress in the body, cortisol is a key indicator in the response to physiological and psychological stress and is reported to be high in children with caries.[1,11] Stress can increase the likelihood of dental plaque formation and inactivate the immune system. As a result, salivary immunoglobulins, especially S-IgA, are inhibited. Stress acts on plasma cells to reduce the production of S-IgA, in addition to other

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antimicrobial proteins in saliva and catecholamines. Eventually, this cycle increases cariogenic bacteria in the oral environment, the saliva is affected, and biofilm binds and penetrates the teeth. Biofilm will increase on the surface of the tooth and caries will occur.[12] Some physiological aspects of immune responses to physical activity and the extent to which cortisol and S-IgA are altered in stressful exercise situations are still unknown.[13,14] Although S-IgA is a major factor in the immune system, its role in caries has not yet been conclusively proven, and the extent to which it is associated with exercise is still debated.[3,15] In some studies, exercise has been suggested as a factor altering salivary cortisol and S-IgA levels, and in recent years the importance of the hormone cortisol in dental caries has been studied. However, some physiological aspects of immune responses during physical activity and the extent to which cortisol changes during stressful exercise are still unknown.[16,17] In some studies, S-IgA and cortisol increased after exercise,[13,18,19] in other studies their decrease was reported after exercise,[9,14] and in some cases no change was observed.[4,14,20] Intensity, duration, and frequency of physical activity appear to affect the complexity of the relationship between changes in salivary cortisol and S-IgA during exercise.[21] Physical activity is divided into beginner and professional categories in terms of intensity, duration, repetition, oxygen consumption, and heart rate. During exercise having maximum oxygen consumption of 80% and maximum heart rate of 90% plays an important role in changes in cortisol and S-IgA levels.[22–24] Therefore, considering that swimming can change oxygen consumption and heart rate,[24] it can be used to study such changes in the saliva. There are fewer studies on the immunological and hormonal systems of child athletes than on adolescents and adults, and due to the contradictory results of previous studies, this study was conducted to investigate the effect of exercise on S-IgA and salivary cortisol in child athletes.

Methods

This cross-sectional study was conducted on boys 6-12 years old. Using statistical methods, the sample size of 43 people in each of the competitive and non- competitive groups was calculated. Considering 10% possibility of dropout, finally 48 people were assigned to each group. The only pool with a professional children's swimming team in Zanjan was Amjadieh pool, so all the sampling steps were done in this center. After coordinating with the pool authorities and the children's swimming instructors, they were asked to invite all the 115 boys to the pool along with one of the parents to attend the study. There were 109 children at the pool on the appointed day. At the beginning of the introductory session, after talking with the educator, 13 children were excluded from the study due to history of acute or chronic illness, allergies, stress and anxiety,[25] respiratory infections, fever, or taking antibiotics in the past 3 weeks. Children with any kind of medical history and those using certain drugs were not included in the study. The study conditions were explained orally for the remaining 96 children and their parents, and child's history, including regular exercise history, number of times they exercised per week, and how many hours each time, was entered in a previously prepared questionnaire. The Beck Anxiety Inventory (BAI) Scale was also used to assess children's anxiety. This questionnaire had been standardized, and its reliability had been reported to be 92%.[26] According to this questionnaire, anxiety is divided into four categories: None (0–7), mild (8–15), moderate (16–25), and high (26–63) anxiety. Using the group matching method, the levels of the boys' anxiety in the two study groups were matched to eliminate its role as a possible parameter from the study.[26,27].Based on the information obtained, the children were divided into two groups of competitive and non-competitive swimmers. A swimmer who had been involved in this sport for at least 2 years and trained 5 times a week, 2.02 ± 0.09 hours each time, was considered a competitive swimmer, and a swimmer who had been engaged in this sport for less than 2 years and trained once a week, 2.02 ± 0.09 hours every time, was considered a non-competitive swimmer.[4] At the end of the introduction session, written consent was obtained from the parents to perform oral and dental examinations of the children and to take two samples of saliva orally. In the first stage, oral and dental examinations were performed by a trained researcher using disposable gloves, a mirror, and a sterile catheter in normal room light in the trainers' room adjacent to the exercise test site. The caries status data was obtained by examination using the DMFT (decayed, missing, and filled teeth) index on permanent teeth and deft (decayed, exfoliated, and filled teeth) index on deciduous teeth. The assessment that was used during inspection included D (decayed) for the carious teeth, where the dental explorer tip was stuck in the cavity, M (Missing) for extracted teeth due to caries, tooth extraction trace, or presence of root residue, and F (Filling) for restored teeth. The assessment that was used during inspection of deciduous teeth included d (decayed) for carious teeth, e (exfoliated) for the extracted teeth due to caries, tooth extraction trace, or presence of root residue, and f (filling) for restored teeth. then summation was done to obtain the DMFT and deft results and their average values.[28] The children were divided into five groups: very low (deft/DMFT: 0–1.1), low (deft/DMFT: 1.2–2.6), moderate (deft/DMFT: 2.7–4.4), high (deft/DMFT: 4.5–6.5), very high (deft/DMFT: > 6.6).[28] In order to better compare the results, the DMFT variable was matched in the two competitive and non-competitive swimmer groups. For this purpose, group matching was used. The age group was divided into two categories: 6–9 and 9–12. The 6–9 age group is related to the early period of dental mixing, and the 9–12 age group is related to the late mixed dentition period. To avoid the effect of the inflammatory process caused by loose and growing teeth during the 9–12 years period,[28,29] which could affect our study results, these two age groups were analyzed separately. Due to the age group of children and the small number of children in relation to the required sample size, it was not possible to match the age between the two study groups, and, finally, there were 48 children in each study group. In the second step, the children were asked to abstain from eating and drinking for one hour. After one hour, each of them was given sugar-free chewing gums, which were chewed for 30 seconds, and then after removing the gum, a 2-cc sample of stimulated saliva was collected in 15 mL sterile tubes. The samples were then placed in an ice-box containing ice. Then, under the supervision of a swimming instructor, a 100-m front crawl exercise test was performed. To warm up, all the children swam 100 meters as a coordinated front crawl, and, then, the main test was performed as a 400-m front crawl. After the exercise test, the second sample of saliva was collected under the same conditions as before. Sampling was done between 5 and 7 PM. The samples were immediately transferred to the pathology department of Ayatollah Mousavi hospital in Zanjan. After centrifugation at 12000 × g, all samples were stored at -20°C until the day of the experiment. Saliva samples were examined for IgA and cortisol by ELISA method. ELISA kit for human samples from Hangzhou Eastpaharm Company (LOT number E20170925) with an accuracy of 0.5 μg/mL and 0.12 ng/mL, respectively, according to the instructions of the kits. Finally, the 43 children in each data group were analyzed using SPSS software version 16. For this purpose, independent *t* test, paired *t* test, and chi-square test were used.

Results

Eighty-six male children in two groups of competitive and non-competitive swimmers participated in this study. First, chi-square test was used to observe the confounding variables of age, anxiety, and DMFT. According to the results, DMFT distribution and anxiety were the same in the two study groups, but the age variable was significantly different in the two groups (Table 1). In both age groups, the pre-training mean of S-IgA and cortisol were not significantly different between the competitive and non-competitive groups. The mean S-IgA in both age groups was higher in the competitive than non-competitive groups, and it increased after training, but not significantly. In the 6–9 age group, the amount of cortisol after training increased in both competitive and non-competitive swimmers, but this increase was statistically significant in the non-competitive group ($P = 0.048$). In the 9–12 age group, the amount of cortisol decreased after training in the competitive group and increased in the non-competitive group, but not significantly (Table 2).

Table 1. Distribution of DMFT, age, and anxiety among competitive and non-competitive swimmers

	Study groups		
	Competitive swimmers (2.02 ± 0.09 hours, 5 times/ week)	**Non-competitive swimmers (2.03 ± 0.18 hours/week)**	***P* value***
deft/DMFT			
0–1.1	10	8	0.604
1.2–2.6	13	11	
2.7–4.4	11	12	
4.5–6.5	6	8	
>6.6	3	4	
Age groups			
6-9	7	21	0.002
9-12	36	22	
Anxiety			
No anxiety (0–7)	26	29	0.549
Mild anxiety (8–15)	14	13	
Moderate anxiety (16–25)	3	1	
High anxiety (26–63)	0	0	

* Chi-square test.

Discussion

Due to the complexity of the effect of exercise on changes in salivary cortisol and S-IgA levels[21] and insufficient information on the immune and hormonal systems in pediatric athletes, this study was performed to investigate the relationship between exercise and two important factors in the development of pediatric dental caries. The S-IgA of the competitive group increased by 28% and 2% in the 6–9 and 9–12 age groups, respectively. In the non-competitive group, the increase of S-IgA was 2% and 12% in the 6–9 and 9–12 age groups, respectively. In studies performed on 5–15-year-old swimmers[13] and 18-year-old athletes,[19] the concentration of S-IgA also increased after exercise testing, but the increase was not significant. One year of continuous exercise (endurance and resistance) of the elderly with moderate intensity[30] and 12 weeks of regular and moderate aerobic exercise training in adults caused a significant increase in S-IgA[31], which can be due to the duration of the study, the intensity of exercise, or the age of the athletes. On the other hand, intense and prolonged exercise in 21-year-old men could not significantly change the concentration of S-IgA in saliva.[9] Twelve weeks of gymnastics in female athlete girls[16] and short periods of high-intensity cycling in 15-year-old boys[8] reduced S-IgA levels after exercise, but these changes were not significant. S-IgA levels decreased significantly after professional swimming in both competitive and non-competitive child professional swimmers[4] and also after intense training by basketball

Table 2. Comparison of IgAs and Cortisol levels in pre- and post-training, for the competitive and non-competitive swimmers in each age groups

Age groups	IgA and cortisol levels	Study groups	N	Mean (Std. Deviation)		Differences (post-pre)	% Change	*P* value
				Pre-training	Post- training			
6–9	S-IgA	Competitive swimmers	7	148.14 (55.88)	190.67 (186.07)	+42.53	↑28	0.590*
		Non-competitive Swimmers	21	144.58 (77.49)	148.69 (74.75)	+4.11	↑2	0.799*
		P value		0.912**	0.394**			
	Cortisol	Competitive swimmers	7	47.00 (10.14)	92.66 (72.59)	+45.66	↑97	0.170*
		Non-competitive Swimmers	21	52.73 (29.88)	66.59 (23.39)	+13.86	↑26	0.048*
		P value		0.626**	0.137**			
9–12	S-IgA	Competitive swimmers	36	205.50 (157.88)	210.00 (194.97)	+4.5	↑2	0.893*
		Non-competitive Swimmers	22	162.27 (114.18)	181.93 (102.18)	+19.66	↑12	0.358*
		P value		0.269**	0.535**			
	Cortisol	Competitive swimmers	36	72.55 (10.14)	69.76 (72.59)	-2.79	↓3	0.814*
		Non-competitive Swimmers	22	61.66 (29.88)	73.11 (23.39)	+11.45	↑18	0.354*
		P-value		0.407**	0.825**			

* Result of paired t-test in order to compare before-after differences.
** Result of independent t-test in order to compare S-IgA and cortisol levels.

athletes.[32] In some studies,[4] such as the present study, S-IgA levels were generally higher in athletes, while the review study by Gleeson et al. found that the concentration of S-IgA in non-athletes was higher than athletes due to the intensity of training and the reverse result.[21] It seems that due to the high dispersion of data, in order to reach a statistically significant level, a higher sample size was needed so that we could speak more decisively about the increase in S-IgA. Gender, DMFT, age, sampling time, and pediatric stress were controlled in this study, but it seems that other factors such as circadian rhythm,[19] seasonal changes,[33,34] and individual nutrition[35] may have also affected S-IgA concentration. Threshold and severe stimulation of the sympathetic system,[9] J curve,[31] ratio of IgA to salivary protein, or ratio of IgA to osmolality [8] can be checked before and after exercise in order to assess the significance of the difference in S-IgA levels. Immunological agents prevent caries by inhibiting the attachment of microorganisms to the tooth surface.[2,9] The role of some of these factors such as S-IgA and cortisol in caries has not yet been conclusively proven, and the extent of change and the relationship between this factor and exercise are still debated.[3,36] The amount of cortisol in the present study increased by 45% in competitive swimmers in the 9–6 age group and decreased by 2% in the 9–12 age group. In non-competitive 6–9-year-old swimmers, the amount of cortisol increased significantly by 13% while in the 9–12 age group it only increased by 11%. The duration of exercise,[9] the correct size of the initial level of cortisol achieved by not eating and drinking for 3 hours,[8] and the intensity of exercise[19] have been identified as factors affecting cortisol changes. Factors influencing the rate of cortisol changes include the role of circadian rhythm through the HPA pathway and the role of cortisol in gluconeogenesis.[19] Exercise for up to 20 minutes with all three intensities of low, medium, and high in terms of oxygen consumption reduces anxiety and salivary cortisol, and an increase in it will occur only after 40 minutes of intense exercise. In fact, exercising for less than 40 minutes will not be able to elicit a response from the HPA system.[20] Gleeson et al[21] Also concluded in their study that 40% oxygen consumption (Vo2max) in exercise not only does not have the ability to increase salivary cortisol but may even reduce it. Increase in cortisol has been reported in sports with 60% and 80% oxygen consumption.[22]

Strengths and Limitations

One of the limitations of the present study was obtaining the consent of the studied samples. The researcher first solved this problem by talking to the officials of the stadium and then holding a briefing session for the parents. The high cost of the required materials and difficulty of access to them due to existing sanctions were other limitations of the study, and the researcher was not able to follow up the samples for a longer time due to financial problems.

Conclusion

Exercise training seems to have more long-term effects on S-IgA because in both age groups)6–9 and 9–12), the amount was higher in competitive than non-competitive swimmers, and after swimming exercise, this amount increased in both groups. Changes in cortisol level in both age groups among competitive and non-competitive swimmers were more complex than those of S-IgA. Doing exercise in the form of regular training sessions can be beneficial. Measurement of immunoglobulin flow rate per minute and *Streptococcus mutans*-specific IgA can be a guide to a better understanding of the relationship between caries and salivary factors. To better understand the relationship between caries and salivary factors in children, it is recommended that the samples be followed up in three, six, or more months in future studies. Also, measuring the flow of immunoglobulin and IgA specific to *S. mutans* can be considered in future studies.

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Authors' Contribution

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Competing Interests

The authors have declared that no conflict of interest exists.

Data Availability Statement

The requests sent to the responsible author's email regarding access to the data of the article will be answered and this access is possible up on the request.

Ethical Approval

This study with the code ZUMS.REC.1396.91 has been approved by the ethics committee of Zanjan University of Medical Sciences.

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