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Caries Experience and Salivary Heat Shock Proteins in Children: A Study in Humid Iraqi Marshlands

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Abstract:

This comparative cross-sectional research aims to evaluate the salivary heat shock protein association with dental caries experience in primary school students living in humid Iraqi Marshlands area compared to their peers in Al-Nasiriyah city. 400 students (6–9 years old) were volunteered in the study. A significant difference in salivary factors and prevalence of dental caries among the two groups were noticed. Also, Marshland's students showed a significantly lower dental caries in comparison to those from Al-Nasiriyah. It reflects the effect of dietary and environmental features on oral health. As well, Marshlands group shows a higher level of salivary pH demonstrating a more alkaline salivary environment which may contribute to caries risk reduction. The outcomes highlight the importance of understanding the interaction between dietary, environmental, and oral health factors. Further research exploring the relationships between salivary indicators and dental caries experience as well as potential interventions to enhance oral health in at-risk populations are recommended.

Keywords: Marshes, Dental caries, Salivary heat shock protein

Introduction:

The lower part of the Mesopotamian basin, where the Tigris and Euphrates owe, is home to the Iraqi Marshlands, one of the largest wetlands in the world, spanning 15,000 to 20,000 square kilometers. Three significant southern cities—Nasiriyah to the west, Amarah to the northeast, and Basra to the south—enclose it, forming a sizable triangle (Al-Mudaffar et al., 2016). The marshes have a humid, subtropical climate (Al-Nasrawi et al., 2021). In humid weather, there is a lot of water in the atmosphere. Sweating is therefore less effective because it is difficult for perspiration to evaporate into the atmosphere. As a result, the body attempts to cool down, which leads to increased perspiration and, ultimately, dehydration. Because dehydration lowers salivary flow,

plaque and oral bacteria accumulate in the mouth. Tooth decay and other dental health issues are caused by an increase in oral bacteria (Sharma et al., 2014) The kind of chronic oral disease that mostly harms the health of humans is dental caries (Cheng Le, et al., 2022),One of the most prevalent dental health issues that individuals now face worldwide is dental caries It is a chronic infectious disease associated with the interplay of behavioral, biological, and socioeconomic factors (Hassan & Athab, 2024) Although progress in oral health, there stays oral health problems globally due to it being widely infectious and spread (Bakhsh, et al., 2021; Al-Obaidi, et al., 2019).

Molecular chaperones known as heat shock proteins (HSPs) aid in preserving protein stability in the face of oxidative stress, heat, exercise, gravity, and calcium ion (Ca2+) variations (Zhao et al., 2020). Heat shock proteins (HSPs) are quickly upregulated when cells are exposed to stressors such as high temperature, inflammation and infection to help cells survive and maintain protein structure and function (Hu et al 2022). They are classified into several families based on their molecular weight and function. The regulation of HSPs is a dynamic and complex process that responds precisely to physiological and environmental changes within the cell. This occurs following exposure of cells to stressors that result in the accumulation of misfolded or damaged proteins (Gomez-Pastor et al., 2018).

They are immunologic adjuvants (Sedlacek et al., 2021). HSP10 family proteins are adjuvant which activate effector cells and induce immune responses (Zhang et al., 2021).

Heat shock proteins greatly contribute to the survival of cariogenic bacteria, e.g., Streptococcus mutans, against acid pH and oxidative stress. Under these stresses, proteins are overexpressed that ultimately promote bacterial survival and biofilm formation, contributing to the development of dental caries (Zhang et al., 2023). The aim of this study was to assess the relationship between levels of HSP in saliva and dental caries in schoolchildren living in humid conditions (marshes) compared to schoolchildren living in the city center.

Methods:

Cross-sectional comparative study design was employed. This study was conducted among primary school students with an age range between 6–9 years in two areas of Thi-Qar Governorate: Al-Ahwar region and Al-Nasiriyah city. The study was carried out over a period of three and a half months, from December 15th, 2024 to March 31st, 2025.

Parents of the children received an information sheet that described the aim and importance of the study in simple terms to ensure voluntary participation.

The sample:

The sample of the present study consisted of primary school students aged 6–9 years. The age of the sample was recorded in accordance to the criteria of the World Health Organization (WHO, 2013), based on the last birthday. According to the information obtained from the General Directorate of Education of Thi-Qar Governorate, the study population included all primary school children within the targeted age group in both Al-Ahwar region and Al-Nasiriyah city.

The representative sample size was calculated using G*Power software version 3.1.9.7 (Program written by Franz Faul, University of Kiel, Germany). With 85% power, alpha error

probability of 0.05 (two-sided), and an effect size of Cohen's D = 0.3 (small effect size), the required sample size was approximately 400 subjects, divided equally into two groups (200 students from Al-Ahwar and 200 students from Al-Nasiriyah). The Cohen's D effect size scale ranges from 0.3 (small), 0.5 (medium), and 0.8 (large). Both male and female students were included in the study sample, and the selection of schools was done randomly (simple random) to represent different geographical areas of the two regions. Children with any systemic diseases or special health conditions that may affect oral health were omitted from the investigation.

Oral examination was used for the procedure, which was conducted in accordance with the WHO 2013 oral health survey's core methodology. Every tooth's four surfaces were inspected. Every tooth was included. Dental mirrors and dental probes are used for clinical assessments. Then collected Unstimulated saliva from the children was collected by allowing them to drool passively into a collection tube over a five-minute period, following the guidelines from the University of Southern California School of Dentistry Prior to collection, the children were instructed to rinse their mouths several times with distilled water and then relax quietly for five minutes. They were then guided to minimize movements, especially around the mouth, and to tilt their heads slightly forward with their mouths slightly open, allowing saliva to flow naturally into the collection tube for the duration of five minutes. (Navazesh and Kumar, 2008). When saliva collection was finished and foam disappeared, the flow rate of saliva was detected by dividing all collections of saliva measured by milliliters by the number of minutes consumed in the collection which was fixed (5 min) expressed by ml/min. Flow rate = Volume (ml) / Time (min). Additionally, saliva, by integrating the pH meter's sensor into the salivary tube and tilting the tube so that the saliva covered the full sensor's surface, the pH was determined. A cooling box containing saliva was transported to the lab. Then 10 minutes centrifuging at 3000 rpm. Then use a micropipette to separate the supernatant and deep freezing (-20C) put it. Using an ELISA kit for determent concentration of salivary heat shock proteins

Result:

The present study is cross-sectional comparative study. The sample of include 400 primary school students aged 6-9 years, 200 students lives in Marshes compared with 200 students that lives in Nasiriyah city. Distribution of sample according to age and gender are displayed in Table 1 this table shows no significant association between age and gender with both groups (Nasiryah and Marshes group).

Table 1. Distribution of student by age and gender among groups.

			Marshes		Nasiriyah		Chi square	p value
			N.	%	N.	%		
Total		6	30	15	30	15	1.538	0.673
		7	40	20	40	20		
		8	70	35	60	30		
		9	60	30	70	35		
	Age (years)		200	50	200	50		
Gender		M	88	44	90	27.5	0.04	0.841

	F	112	56	112	72.5		

Table 2 shows a statistical and descriptive comparison of caries occurrence of primary teeth among groups. In terms of (ds), the marshes group recorded a significantly lower mean compared to with a highly significant difference. For (ms), Marshes also showed lower mean but with no significance. Regarding (fs), Marshes had no recorded filled surfaces, while Nassiriyah had a mean of 0.352±0.097 This difference was significant. The overall (dmfs) score was markedly lower in Marshes compared to Nassiriyah, with a highly significant difference. These results indicate that Nassiriyah children show a higher overall experience of dental caries in primary teeth.

Table 2. Statistical and descriptive test of caries experience of primary teeth among groups.

Vars.	Groups				T test	p value
	Marshes		Nassiriyah			
	Mean	±SD	Mean	±SD		
ds	13.483	0.888	21.364	0.844	6.433	**0.000**
ms	1.126	0.240	1.606	0.243	1.405	0.161
fs	000	0.000	.352	0.097	3.631	**0.000**
dmfs	14.609	0.998	14.609	0.895	6.499	**0.000**

Table 3 shows a statistical and descriptive comparison of the caries occurrence of permanent teeth among groups. In terms of (DS), the marshes group recorded a lower mean compared to Nassiriyah with no significant difference. For (MS), there is no missing teeth for both groups. Regarding (FS), Marshes had no recorded filled surfaces, while Nassiriyah had a mean of 0.048±0.028. This difference has no significant. The overall (DMFS) score was lower in Marshes compared to Nassiriyah, with a highly significant difference.

Table 3. Statistical and descriptive test of caries experience of permanent teeth among groups.

Vars.	Marshes		Nassiriyah		T test	p value
	Mean	±SD	Mean	±SD		
DS	0.616	0.152	1.103	0.204	1.913	0.057
MS	.000	0.000	0.00	0.000	---	-----
FS	0.000	0.000	0.048	0.028	1.716	0.088
DMFS	1.616	0.152	1.152	0.209	2.073	**0.039**

Table 4 shows descriptive and statistical comparison of salivary flow rate, PH and heat shock protein among groups. The mean salivary PH was greater in Marshes compared to Nassiriyah group with significant difference, indicating that children in the Marshes group had a more alkaline salivary environment than those in Nassiriyah. For salivary flow rate, marshes had a lower mean flow rate than Nassiriyah, this difference is not a significant. The mean heat shock protein level was higher in Marshes than in Nassiriyah with no significant. The result concludes that the only

significant difference between the groups was observed in salivary PH, where the Marshes group showed a higher mean value.

Table 4. Statistical and descriptive test of salivary parameters among groups.

	Marshes		Nassiriyah		T test	p value
	Mean	±SD	Mean	±SD		
Salivary PH	6.896	0.042	6.580	0.069	4.087	**0.000**
SFR	0.740	0.042	0.825	0.111	0.775	0.440
heat shock protein	1.990	0.135	1.874	0.149	0.577	0.565

Examines the association between dental caries experience and the salivary parameters (SPH, SFR, and HSP) in both groups. In the Nasiriyah group, some positive and negative correlations were noted between caries indices and salivary factors. Notably, HSP showed significant relationships with certain caries indices, suggesting a potential link between stress-related salivary proteins and the occurrence of dental caries. In the Marshes group, the correlations were generally weak and not statistically significant. This may indicate that the role of salivary indicators in relation to caries experience is less pronounced in this population, as shown in Table 5.

Table 5. Relationship between caries experience with salivary parameters

Groups		SPH		SFR		HSP	
		r	p	r	p	r	p
Marshes	ds	-0.111	0.419	-0.130	0.344	0.023	0.866
	dmfs	-0.118	0.390	-0.157	0.253	0.046	0.736
	DS	-0.063	0.649	-0.189	0.166	-0.056	**0.685**
	DMFS	-0.063	0.649	-0.189	0.166	-0.056	**0.685**
Nassiriyah	ds	-0.208	0.175	0.069	0.657	-0.217	0.157
	dmfs	-0.243	0.112	0.053	0.732	-0.185	0.230
	DS	-0.135	0.383	-0.133	0.388	-0.352	**0.019**
	DMFS	-0.135	0.383	-0.133	0.388	-0.352	**0.019**

Discussion:

The outcomes highlight knowledge in the oral health status amongst primary schoolchildren in the Mesopotamian Marshes by comparison to Al-Nasiriyah. The result will indicate substantial differences in dental caries experiences, salivary factors, and heat shock protein (HSP) levels between both groups.

Nutritional factors:

One significant determining factor of the oral health in Marshes is brought by the dietary pattern being followed by its people. Traditionally, the diet in the Marshes consists of locally sourced fish and vegetables, often lacking in processed sugars that contribute to dental caries. Sharma et al.

showed that consumption of dietary patterns based on natural foods may reduce the occurrence of dental caries in children (2014). This is consistent with our results, as the children from the Marshes also showed significantly lower caries experience than children from the city of Al-Nasiriyah, where a more cariogenic, sugar- and processed-foods-rich diet is common.

Salivary pH Levels:

The salivary flow rate was collected as in the previous Iraqi study, (Obied & Ahmed, 2024; Ajeel & Diab, 2024; Ahmed, et al., 2024) and The saliva samples were collected using the spitting method as described by (Hassan & Athab,2024) , Unstimulated saliva plays a vital role in preserving oral health and overall well being of the mouth (Ali & Yas, 2025) The other important result of this study was the greater mean salivary pH in the groups of the Marshes than the Al-Nasiriyah. The reason behind higher pH observed may be due to a special condition observed in the Marshes since the natural foods may play a role in higher saliva condition which is alkaline in nature. Research by (Wolkoff et al. 2021) suggests that a higher salivary pH is capable of neutralizing the acids that oral bacteria create, thereby reducing the risk of dental caries. This may explain why children in the Marshes demonstrated a lower prevalence of caries.

Heat shock pfs roteins:

The study also highlighted variations in heat shock protein levels between the two groups. Higher levels of HSPs in children from the Marshes may indicate a physiological response to environmental stressors, for example, humidity and temperature fluctuations. According to (Hu et al. 2022), HSPs are vital in maintaining cellular integrity and function during stress, which may also influence immune responses in the oral cavity. This suggests that the elevated HSP levels could be a protective mechanism against oral diseases, contributing to the observed differences in caries prevalence Saliva flow rate is the volume of Saliva different during lifespan (Xu, et al., 2018). Stress may connect with several pathologies of the oral cavity (Kadhum, et al., 2020). Oral and dental health including caries may result from the bad impact of stressful life evaluation of health routine give good outcome (Abedi, et al., 2020).

Conclusion:

In conclusion, children from the Mesopotamian Marshes exhibited unique salivary and HSP profiles compared to their peers in Al-Nasiriyah, likely reflecting the influence of environmental stressors, cultural factors, and limited access to preventive care. These results underscore the need to consider both ecological and molecular aspects when developing oral health promotion interventions. Future studies could test larger population sizes and other interventional strategies to possibly include salivary biomarkers as a means for early prevention and health monitoring in at-risk groups.

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of Interest:

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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تجربة تسوّس الأسنان وبروتينات الصدمة الحرارية اللعابية لدى الأطفال: دراسة في مناطق الأهوار الرطبة في العراق

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الملخص:

يهدف هذا البحث المقطعي المقارن إلى تقييم العلاقة بين بروتينات الصدمة الحرارية في اللعاب وتجربة تسوّس الأسنان لدى تلاميذ المدارس الابتدائية المقيمين في مناطق الأهوار العراقية الرطبة، ومقارنتها بنظرائهم في مدينة الناصرية .شملت الدراسة 400طفل تتراوح أعمارهم بين 6–9سنوات شاركوا طوعًا في البحث . أظهرت النتائج وجود اختلاف معنوي في العوامل اللعابية وانتشار تسوّس الأسنان بين المجموعتين .كما أظهر تلاميذ منطقة الأهوار انخفاضًا واضحًا في معدلات تسوّس الأسنان مقارنة بأقرانهم في الناصرية، مما يعكس تأثير العوامل الغذائية والبيئية على صحة الفم .بالإضافة إلى ذلك، أظهرت مجموعة الأهوار مستوى أعلى من الأس الهيدروجيني (pH)اللعابي، ما يشير إلى بيئة فموية أكثر قلوية قد تُسهم في تقليل خطر الإصابة بالتسوّس .تُبرز هذه النتائج أهمية فهم التفاعل بين العوامل الغذائية والبيئية وصحة الفم، كما توصي بإجراء دراسات إضافية لاستكشاف العلاقة بين المؤشرات اللعابية وتجربة تسوّس الأسنان، وتحديد التدخلات الممكنة لتحسين صحة الفم لدى الفئات المعرضة للخطر

الكلمات المفتاحية :الأهوار، تسوّس الأسنان، بروتين الصدمة الحرارية في اللعاب.