"The Prevalence of Recurrent Aphthous Stomatitis in the Armenian Ethnicity"

A Project Submitted to the College of Dentistry, University of Baghdad, Department of Oral Diagnosis in Partial Fulfillment for the Bachelor of Dental Surgery

By:

Talar Nobar Aram

Supervised by:

Assist. Lec. Maryam Hameed Alwan

BDS, MSc (Oral Histology)

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Certification of the Supervisor

I certify that this project entitled "**The Prevalence of Recurrent Aphthous Stomatitis in the Armenia Ethnicity**" was prepared by the fifth-year student *"***Talar Nobar Aram***"* under my supervision at the College of Dentistry/University of Baghdad in partial fulfillment of the graduation requirements for the Bachelor Degree in Dentistry.

Supervisor's name:

Date:

Dedication

I Dedicate This Paper to **The Brave Souls of Artsakh.**

Acknowledgement

First and foremost, praise to **The Lord**, The Almighty for blessing me with sufficient health and knowledge to be able to finish this paper.

I must mention my gratefulness for **My Parents and My Sister** and all of their unconditional support throughout my life.

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Abbreviation	Meaning
RAS	Recurrent Aphthous stomatitis
RAU	Recurrent Aphthous ulcer
MiRAU	Minor Recurrent Aphthous Ulcer
MaRAU	Major Recurrent Aphthous Ulcer
HSV	Herpes Simplex Virus
CBC	Complete Blood Count
TNF	Tumor Necrosis Factor
HLA	Human Leukocyte Antigen

Introduction

Recurrent Aphthous Stomatitis (RAS) belongs to the group of chronic inflammatory diseases of the oral cavity, the characteristic feature of the disease is single or multiple shallow round ulcerations usually occurring in non-keratinized area like the lips, ventral surface of the tongue, floor of the mouth, buccal mucosa and soft palate **(Chattopadhyay, 2007)**. The incidence of RAS ranges from 5% to 50% depending on the ethnic or socioeconomic group **(Akintoye, 2014)**.

Despite all the efforts the exact etiology of RAS is still ambiguous, and both genetic and environmental factors are thought to be indicated, the precipitating factors include stress, physical or chemical trauma, allergies, infection, drugs or nutritional deficiencies. And stress is believed to be a major risk factor for RAS due to its effect on salivary cortisol levels, para-functional habits that lead to self-inflicted trauma and alterations to the immune system **(Albanidou *et al*, 2008)**.

Anxiety is known as the body's response to stress, and there is an ongoing debate over the effects that caffeine consumption has on anxiety, since caffeine is known as the most widely consumed psychostimulant substance in the world **(Lara, 2010)**. Many cited evidence has shown different results, some stated that there was no correlation between caffeine on stress and anxiety **(Hall *et al*, 2015)**, Other research stated that very high doses can increase stress levels while moderate to low doses helped in reducing stress levels **(McLellan *et al*, 2016)**. Since stress is a multifactorial condition, it's better to research specific individuals with similar lifestyles to accurately measure their stress levels and caffeine habits, therefore in

this paper we will focus on the Armenian ethnicity, where genetics and cultural costumes are shared between the subjects.

Aims of the study:

1. Understand the relationship between caffeine, anxiety and RAS.
2. Measure stress levels within the Armenian Ethnicity.
3. Estimate the prevalence of RAS within the Armenian Ethnicity.

Chapter one: Review of Literature

1.1 Definition

The term Aphthae is a derivative of the Greek word (Aphthi), which stands for "to set fire" or 'to inflame" and is thought to have been used first by the renowned philosopher Hippocrates **(Van der wal, 2018)**.

Recurrent Aphthous Stomatitis (RAS) also referred to as Recurrent Aphthous Ulcer (RAU), Stress Ulcer or Canker Sore, is a disorder described as chronic recurrent ulcerations that are confined to the oral cavity, RAS represents the most common oral ulceration, the condition is characterized by one or several shallow, round, painful oral ulcerations that heal spontaneously **(Preeti et al, 2011),** It can be classified into three types according to its size and duration (Major Aphthous Ulcer, Minor Aphthous Ulcer and Herpetiform Aphthous), regardless of the type, RAS is usually confined to non-keratinized epithelium (buccal mucosa, labial mucosa, ventral and lateral surfaces of the tongue, floor of the mouth, soft palate and the oropharynx), however severe cases may involve the keratinized epithelial surface like the hard palate **(Shafer, 2009)**.

Many pathological conditions can cause oral ulcers that might seem similar to RAS including Bahcet's disease, herpes infection, HIV related ulcers, Crohn's disease or ulcerative colitis, with a detailed history, examination and if necessary laboratory testing the clinician can be guided towards the correct diagnosis.

1.2 Epidemiology

The prevalence of RAS has a wide range, in general it effects about 20% of normal individuals **(Kaleswara et al, 2015)**, the incidence is found to vary from 5% to 50% depending on the ethnicity and socioeconomic status of the individuals, therefore the prevalence of RAS is influenced by the population that is being studied, environmental factors and the genetic factors, it's been found that children of RAS-positive parents have a 90% chance of getting RAS compared to the 20% chance of those of RAS-negative parents **(Scully *et al*, 2004),** many studies revealed an increased prevalence of RAS in students specifically of higher levels in education, this finding supports the effect that stress holds on RAS occurrence.

1.3 Classification and Clinical presentations

RAS is presented with well demarcated round recurrent oral ulcerations with yellow or white pseudomembrane and an erythematous halo, the ulcers are painful and disturb the patient while eating and speaking, occasionally a prodromal sensation of burning or tingling may occur **(Chattopadhyay, 2007).** RAS occurs in patients who are otherwise well, the ulceration episodes occur around 3-6 times per year and show no gender predilection, RAS has been classified by Stanley in 1972 based on size, duration and scarring into three groups:

1.3.1 Minor Aphthous

Also called Miculiz's Aphthae or mild Aphthous ulcer, it is the most common type as it constitutes 80% of all RAS cases, MiRAS is characterized by small ulcers 8-10 mm in size, 1 to 5 in number (figure 1), mostly effecting non-keratinized epithelium like labial mucosa, buccal mucosa and floor of the mouth. These ulcers heal within 10-14 days without scarring.

Figure 1: Minor ulcers on the lateral margin of the Tongue and Labial mucosa **(Scully, 2006)**.

1.3.2 Major Aphthous

Also called Sutton's disease or periadenitis mucosa necrotica recurrens, it's considered as a rare type as it constitutes 10%-15% of all RAS cases, MaRAS are more severe than the MiRAS as it can reach (>1cm), deeper, more painful and effects both keratinized and non-keratinized epithelium (figure 2), these ulcers persist for up to 6 weeks and often heal with scarring, dysphagia and fever are common **(Boulinguez *et al*, 2003).**
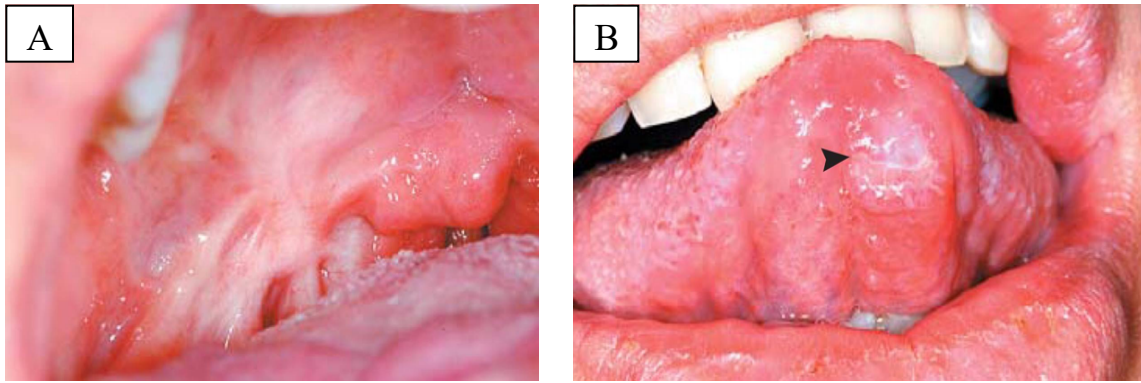
Figure 2: In **A** we see pronounced scarring in the palate after major RAS healed. While in **B** the major RAS is affecting the ventral surface of the tongue **(Scully, 2006)**.

1.3.3 Herpetiform Aphthous

The least common type of RAS, mostly found in adults, the patient presents with multiple crops ranging from 10 to 100 in number, measuring (<5mm) in size scattering over a large area in the oral mucosa usually with marked erythema (figure 3), these ulcers can coalescence to form large ulcers, they heal within 10-14 days and have the potential of scarring **(Rivera-Hidalogo *et al*, 2004),** this type of RAS is commonly mistaken for herpes simplex virus (HSV). Female predilection has been noticed.

Figure 3: Recurrent Herpetiform ulcers **(Nair, 2011)**

1.4 Histopathology

The microscopic picture of Aphthous ulcer is considered to be non-specific therefore the final diagnosis must be made based on clinical examination and history. The mucous membrane of the ulcer area itself shows discontinuity of the epithelium due to necrosis of superficial tissue with fibrinopurulent exudate of clotted fibrin and red blood cells forming a haemorrhagic foci. The epithelial layer is infiltrated with lymphocytes and neutrophils, neutrophils are predominant in the immediately below the ulcer area while in the periphery there is an intense leukocytic infiltration, primarily T-cells. While in older lesions a small portion of plasma cells and eosinophil can be found **(Natah et al, 2004).**

The presence of erythrocytes around the margins of the ulcer with sub epithelial neutrophils, macrophages and non-specific binding of stratum spinsoum cells to the immunoglobulins as a result of vascular leakage and diffusion of serum proteins, are findings that may suggest that the pathogenesis of RAS is mediated by immune complex vasculitis **(Jurge et al, 2006).**
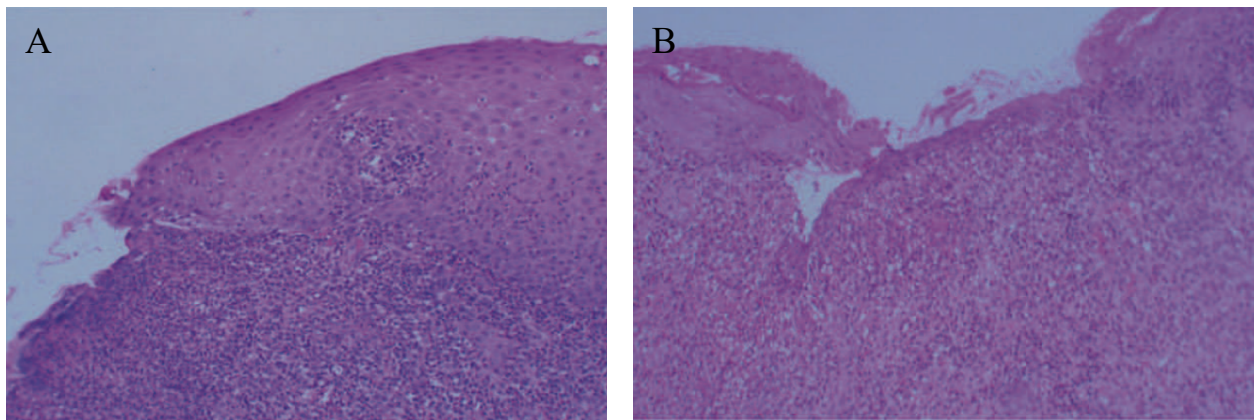
Figure 3: A Note discontinuity of the epithelium and fibrinous exudate. (100x). **B** Infiltration of the connective tissue with inflammatory cells mainly neutrophils and histiocytes, with increased vascularity and perivascular inflammatory infiltrate (200x) **(Messadi and Younai, 2010).**

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1.5 Etiology

The precise etiology of RAS remains unclear, previous literature have shown variable results; however RAS is believed to have a multifactorial etiology, also several local and systemic factors were presumed to be predisposing factors, including:

1.5.1 Trauma

Local trauma can play a role in intuiting mucosal injury and inflammation that leads to ulcers, local trauma can be induced by abrasion during tooth brushing, local anesthesia injection, Laceration by a sharp tooth, chemical burns, thermal injury or cheek biting, however it's important to note that not all traumas lead to RAS. A study conducted by **Wray *et al*, 1981** confirmed that regardless of the mechanism of the injury, trauma to the oral mucosa can cause ulceration in people who are susceptible to RAS, and the simulated ulcers had no histological differences from spontaneous ulcers.

1.5.2 Hereditary factors

This is this best documented factor, in a study by **Scully *et al*, 2004** found that the likelihood of RAS is 90% in patients with parents whom are effected but only 20% when neither parents had RAS history, various associations with HLAs and RAS have been reported specifically; HLA-A2, HLA-B5, HLA-B12, HLA-B44, HLA-B51, HLA-B52, HLA-DR2, HLA-DR7 and HLA-DQ series **(Albanidou *et al*, 2008),** these associations tend to vary with different racial and ethnic groups.

1.5.3 Deficiencies

Hematinic deficiencies were noted to be twice as common within RAS patients **(Kozlak *et al,* 2010),** in a study done by **Koybasi *et al,* 2006** found that 35% of RAS patients had vitamin B12 deficiency, so far it's not clear how vitamin B12 can cause ulcers however the dramatic response to vitamin B12 replacement therapy showed positive results, which suggests a direct role of the vitamin on RAS **(Piskin et al, 2002).** Folic acid, calcium, magnesium, ferritin, phosphorus and hemoglobin were found to have some degree of connection to RAS, but they weren't of any big significance.

1.5.4 Hormonal changes

Different and conflicting studies were made regarding this factor, minor predilection was found towards woman during the onset of their menstrual cycle, and an exacerbation of RAS cases were noticed during puberty. No connection has been noted between RAS and menopause so far **(Ajmal *et al*, 2018).**

1.5.5 Drugs

Certain medications have been associated with the development of RAS like lesions including Nicorandil (a potassium channel activator for treatment of ischemic heart condition), beta blockers and anti-inflammatory drugs (like propionic acid, diclofenac and piroxicam) therefore it's important to take a detailed medical history from the patient of frequent RAS in order to identify any possible predisposing factors **(Tarakji *et al*, 2015)** .

1.5.6 Allergy

Hypersensitivity to some medications, restorative materials and certain food substances (like cow's milk, gluten, nuts, and chocolate), have been considered as a possible RAS risk factor, as there is some association between IgE antibodies and clinical manifestations of RAS **(Besu *et al*, 2009).**

1.5.7 Stress and Anxiety

Psychological stress has been presumed as trigger for RAS, especially since stress prompts para-functional habits like lip and check biting. The majority of literature have shown that the peak of RAS was within students of higher education who face a great deal of stress in their daily life. In a study done by **Gallo *et al*, 2009**, the result showed that 68% of patients reported the occurrence of RAS was associated with particular situations such as a new job, new family problems or during exam periods.

1.6 Diagnosis

Due to the absence of a definitive etiology or diagnostic tests for RAS, the final diagnosis is essentially made by exclusion of other pathologies that can cause recurrent oral ulcers.

Detailed history, thorough examination and when necessary, laboratory tests should distinguish RAS from other lesions such as erythema multiform, pemphigus, pemphigoid, drug reactions or viral stomatitis. The history process should include the following:

- Obtaining symptoms that suggest underlying medical conditions such as HIV.

- Any connective tissue disease that links to lupus erythematous.

- Any gastrointestinal discomfort suggesting inflammatory bowel disease.

- Any skin involvements specifically in the genitals or the eye region suggesting Bahcet's Syndrome.

A diagnostic criteria was suggested by **Natah *et al*, 2004**, they proposed that the diagnosis of primary RAS minor (idiopathic) or the secondary RAS minor (associated with systemic disease) can be made when the condition fulfill four major criteria (see Table 1) plus at least on minor criteria (see Table 2).

Table 1: Major criteria for diagnosing the condition as RAS

Major criteria	Description
1- external appearance	Single or multiple round/oval shaped ulcers, never preceded by vesicles. The ulcers are shallow and have regular margins and a yellow–grey base surrounded by thin erythematous halos. Variable in size, but less than 1 cm in diameter.
2- Recurrence	At least three attacks of RAS within the past 3 years and the recurrences do not affect the same focal site.
3- Mechanical hyperalgesia	The lesion is painful, and the pain is exacerbated by movement of the area affected by the ulcer.
4- Self-limitation	The ulcer heals spontaneously without sequelae either with or without treatment.

* Adopted from: Int. J. Oral Maxillofac. Surg. 2004; 33: p 227

Table 2: Minor criteria for diagnosing the condition as RAS

Minor criteria	Description
1- Family	A positive family history of RAS as present.
2- Age of onset	The first RAS attack started before the age of 40.
3- Location of ulcers	Occur in non-keratinized oral mucosa.
4- Duration of the lesion	Each bout of ulceration lasts from few days to two weeks.
5- Pattern of recurrence	Irregular.
6- Histological examination	Shows non-specific inflammation.
7- Presence of precipitating factor	The attacks are triggered by hormonal changes, exposure to certain foods or drugs, infections, stress or local trauma.
8- Presence of hematinic deficiencies	Laboratory investigations reveal an accompanying hematinic deficiency. In particular, ferritin, folate, iron, vitamin B and zinc.
9- Negative association with smoking	RAS patient is a non-smoker or develops the ulcer after stopping smoking.
10- Therapeutic trial with gluco-corticosteroids	Positive response to treatment with local or systemic steroid.

* Adopted from: Int. J. Oral Maxillofac. Surg. 2004; 33: p 227

1.7 Management

No curative therapy for RAS is available due to the mostly mild nature of the condition and not being able to outline the exact etiology behind it. Many patients feel better when practicing good oral hygiene and occasionally palliative treatment may be necessary for patients that face multiple outbreaks in a short period which interferes with their ability to masticate or speak.

The first step for RAS management should involve identifying and treating any modifiable predisposing factor, for example in patients with frequent ulcers hematological tests might be indicated, including testing CBC, folate, serum ferritin

and vitamin B12 levels, in case of a positive result the clinician should make the appropriate referral **(Messadi and Younai, 2010).** The patient should also be informed of the effects of bad habits (such as cheek biting) and stress on RAS to attempt to reduce them. It's advised for patient to avoid salty, crusty and spicy food to avoid irritating the ulcer any further.

Although it's not possible to prevent RAS, our aim is to lessen the symptoms by reducing the number, size or frequency of the ulcers, many different approaches have been suggesting depending on the severity of the case, including the following:

1.7.1 Topical therapy

This approach is effective in most RAS patients, but alone it can't prevent the formation of new lesions and it's not adequate for severe cases.

Topical anesthesia, such as lidocaine (Anginovag, Xylocaine sprays) or benzydamine are widely used as they're cheap, safe and effective in pain relief, usually applied 4 times a day till the ulcer is healed.

Topical corticosteroids may reduce pain and speed healing as they tend limit the inflammatory process, however they aren't readily available in oral pastes and won't remain on the oral mucosa for long therefore the patient is instructed to avoid eating and drinking for minimum of 30 minutes after the application. Steroid rinses of 0.1% or 0.2% triamcinolone, 0.3% hydrocortisone and dexamethasone are effective if the patient has diffuse ulcers that cover a large area, this rinse is used with caution on limited cases only as it has various side effects. An intralesional injection of

triamcinolone (0.1-0.5 ml per lesion) can also be considered for deep painful lesions **(Messadi and Younai, 2010).**

1.7.2 Mouthwash

Mouthwashes that contain Chlorhexidine improve oral hygiene by inhibiting bacterial accumulation on the tooth surface, this antimicrobial agent has been found to reduce the number of ulcer days and increase the duration of ulcer free intervals **(Suharyani *et al*, 2021)**. The shortcoming of this approach is that regular Chlorhexidine use can lead to undesired exogenous dental staining, and it has a bitter taste.

1.7.3 Systemic therapy

When severe cases don't show improvement after topical therapy, a systemic therapy is considered. Drugs such as colchicine, thalidomide, prednisone and dapsone have been effective but the clinician must weigh the potential benefits against the risks before administration.

Systemic corticosteroids such as oral prednisone has shown effectiveness in severe RAS cases, but long-term use has adverse effects like depression, hyperglycemia, osteoporosis and moon face.

Colchicine reduces the phagocytic function of neutrophils which decreases the number and duration of RAS in most cases, an open trial done by **Fontes and Huttenberg, 2002** has shown symptomatic improvement in 63% of the cases within only 3 months of therapy. Adverse effects of this drug include diarrhea, nausea,

headaches and abdominal discomfort. Also, this drug is contraindicated for pregnant woman.

Thalidomide TNF-alpha inhibitor is used in extreme cases only (usually with HIV patients), a 4-week course of 20 mg thalidomide showed significant improvements in pain management, however this drug is potentially toxic and associated with severe life-threatening birth defects, therefore patients receiving this drug should use two different birth control methods and take pregnancy tests monthly, other side effects may include peripheral neuropathy and abdominal discomfort. Thalidomide is prescribed only when all other less toxic approaches fail to work **(Scully, 2006)**.

1.7.4 Home remedies

The most prevalent and accepted home remedy for RAS is gargling with salt water, as salt has an antiseptic effect that soothes the pain and provides comfort.

Several claims have been made towards the effect of natural products in reducing RAS symptoms such as honey, tahini, coconut oil, clove oil, turmeric powder, pomegranate, yeast, garlic, Aloe Vera, baking soda or apple cider juice.

Due to lack of education people aren't aware that RAS can heal spontaneously within days and might assume that the ulcer healed as a result of a home remedy that they've tried and begin to advocate false practices. A study done by **Sawair, 2010** attempted to determine the type and prevalence of treatments used by patients for RAS, it was found that some patients were using antifungals, antivirals and even cigarette ash to treat RAS because they were following a relative's advice, this study highlighted the importance of patient education (see Table 3).

In recent years with the advancement of technology, social media created a platform for everyone to share their opinions and recommendations, it only takes a couple of seconds to type in your symptoms and get thousands of suggestions on how to control them, of course most of these suggestions are personal and not scientific therefore caution and further research should be taken before attempting them.

Table 3: Information source for RAS treatment in patients from Sawair's study

Treatment	Him/herself	Friends	Relatives	Pharmacist	GDP	OM	MP	Total
Alternative medicine	8 (9.30)	4 (4.7)	68 (79.1)	2 (2.3)	1 (1.2)	0 (0)	3 (3.5)	86 (100)
Conventional medicine	16 (8.8)	3 (1.7)	51 (28.2)	29 (16)	22 (12.2)	2 (1.1)	58 (32)	181 (100)
Total	24 (9.0)	7 (2.6)	119 (44.6)	31 (11.6)	23 (8.6)	2 (0.7)	61 (22.9)	267 (100)

GDP, general dental practitioner, OM, oral medicine specialist, MP, medical practitioner.

* Adopted from: J. of Alternative and Complementary Medicine Volume 16, 2010, p. 653

1.8   Caffeine

Caffeine is the most widely consumed psychostimulant substance in the world as almost all caffeine intake comes from dietary sources, studies have shown that chronic intake has only minor negative effects therefore governmental agencies imposed no regulations on its use. Caffeine has both molecular and neural effects on the human body, especially due to its primary effect as an adenosine receptor antagonist that leads to important secondary effects on other neurotransmitters like dopamine, serotonin, noradrenaline, acetylcholine and glutamate **(Kennedy *et al*, 2022).**

1.8.1 Metabolism

When ingested orally, caffeine is rapidly absorbed and distributed throughout the total body water, it reaches its peak plasma level between 30-75 minutes, the half-life of caffeine is around 5 hours, but it can increase with higher intake or with liver impairment, and it's reduced in cigarette smokers **(Mandel, 2002).**

Children are more susceptible to caffeine due to their small size, as the half-life of caffeine in their body would be around 4 days. The medullary, vasomotor, vagal and respiratory centers are all stimulated by caffeine, due to an increased sensitization to carbon dioxide.

1.8.2 Effects of caffeine

According to previous literature caffeine had beneficial effects on psychomotor speed, alertness and haste of processing new stimuli even when caffeine was consumed in low doses **(McLellan *et al*, 2016),** large doses can cause tachycardia, arrhythmias and has unpredictable effects on blood pressure.

Caffeine has a potent effect on psychological activity as it alters behavior, it can affect sleeping habits, task-oriented performance, fatigue, attention span, and these effects are beneficial when low doses are consumed however consuming more than 1g of caffeine orally can cause adverse effect and a 10g dose may even be lethal **(Mandel, 2002).**

Caffeine increases dopamine transmitters that are known as the "reward center", therefore a moderate dose of 75-150 mg of caffeine can lead to happiness, calmness and clear headiness, these symptoms reduce stress effects in healthy individuals.

In a study done by **Klevart, 2022** it was stated that consumption of 5 cups of coffee is anxiogenic and it increases heart rate due to the peripheral adenosine antagonism that can contribute into panic attacks.

1.8.3 Caffeine habits within the Armenian ethnicity

Armenia doesn't have any role in growing coffee nor producing it however it's considered as a large importer for a small country, in a comparison done between 159 countries by the Helgi library it was found that Armenia consumed 3.83 Kg of coffee per capita in 2018. In 2011 alone Armenia imported over 16 million Kg of coffee while it had less than 3 million citizens, this highlights their heavily caffeinated lifestyle.

This habit goes back it time, in fact in 1685 the first registered coffee house in Vienna was opened by an Armenian called Hovhannes Astvatsatour (also known as Johannes Diodato), this coffee shop was one of the first in Europe and helped share the coffee culture that the Armenians had and normalizing it as a daily routine among the Europeans. Daily consumption of coffee in various age groups is a practice that Armenians associate with their routine life, coffee is a must with company, celebrations and for a peaceful afternoon rest, therefore it is expected that the above-mentioned effects of chronic caffeinating on the body to be vibrant in the Armenian ethnicity.

Coffee consumption is a popular cultural habit in Armenia, and some studies suggest that social factors and cultural norms may play a role in the high prevalence of coffee consumption among Armenians. For example, a study by **Grigoryan *et al,* 2018** found that social interactions and traditions associated with drinking tea and coffee were important factors in the dietary patterns of the Armenian population.

A study conducted by **Petrosyan *et al,* 2015** aimed to assess the prevalence of cardiovascular risk factors among Armenian adults. The study included 864 participants aged between 25-64 years and found that 96% of participants reported consuming caffeinated beverages, including coffee and tea. The average daily intake of coffee was 100 ml, which is equivalent to approximately one small cup of coffee. The study concluded that the prevalence of caffeine consumption among Armenian adults is relatively high. Similarly, a study conducted among the Armenian population by **Khammour *et al,* 2019** found that 78% of participants reported drinking coffee, and the average daily coffee consumption was 157 ml. which was considered quite high.

Chapter Two: Subjects, Material and Methods

2.1 Sample Collection

Online survey data were collected from subjects of Armenian ethnicity, the subjects were asked to participate on voluntary basis, all the participants were fully informed about the aims of the study and confidentially of the data, they were also assured that the data would be used only for research purposes and that no name or IP address was being recorded to protect their privacy and protect the confidentiality of their personal informations.

The study sample consisted of 97 subjects, 21 males and 76 females, all of Armenian ethnicity, the self-administered questionnaire was conducted using the Google Forms platform and distributed to the participants by various social media applications during the period of January 2023.

Two online forms were created with identical questions but in different languages, the first form was in English and the second in Armenian, in order to accommodate for the language preferences of the participants and make sure the questions were clear and understood for everyone.

2.2 Methods

The questionnaire depended on three parts:
1. Caffeine consumption assessment.
2. The perceived stress scale (PSS).
3. RAS frequency assessment.

2.2.1 Caffeine Consumption Assessment

This part included seven questions regarding caffeine habits, these questions assess the frequency, type and amount of caffeine intake in order to evaluate the caffeine habits within the Armenian ethnicity, the questions and options are listed below:

Table 4: Caffeine consumption related questions and options.

Questions	Options
Sources of caffeine	• Coffee • Tea • Caffeinated soft drinks • Energy Drinks • Chocolate • Others: _____
How often do you consume Caffeinated beverages?	• Once a day • 2-3 times daily • 4-5 times daily • More than 5 daily • 1-3 times weekly • 4-6 times weekly
What time of the day do you usually consume caffeine ?	• Morning • Afternoon • Evening
When do you prefer to have caffeine?	• Before eating • While eating • After eating

At what age did you start taking caffeine?	- 13 – 17 - 8 - 12 - Over 18 - Under 8
Caffeine makes me	- Alert, energized and less sleepy - Think clearly, pay more attention and sharpen my memory - Relax, calm down, and improves my mood
Cup size	- S (240 ml) - M (360 ml) - L (480 ml) - XL (600 ml)

2.2.2 Perceived Stress Scale

The PSS is a widely used psychological instrument for measuring the personal perception of stress, the questions of PSS are of general nature and free of content specificity as they assess the feeling and thoughts of the subject during the last month, in each question the respondents are asked how often they felt a certain way. For each question we use a scale ranging from (0-5) where 0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often and 4 = very often. The questions are:

1. In the last month, how often have you been upset because of something that happened unexpectedly?

2. In the last month, how often have you felt that you were unable to control the important things in your life?

3. In the last month, how often have you felt nervous and stressed?

4. In the last month, how often have you felt confident about your ability to handle your personal problems?

5. In the last month, how often have you felt that things were going your way?

6. In the last month, how often have you found that you could not cope with all the things that you had to do?

7. In the last month, how often have you been able to control irritations in your life?

8. In the last month, how often have you felt that you were on top of things?

9. In the last month, how often have you been angered because of things that happened that were outside of your control?

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

Determining PSS score is done by reversing the scores for questions 4, 5, 7, and 8. On these 4 questions, change the scores like this: $0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0$. Then add up scores for each item to get a total, scores can range from 0 to 40 with higher scores indicating higher stress levels.

The PSS is important because two individuals could have the exact same events and experiences in their lives during the same time period, yet depending on their perception, the total score could put each one of them in a different stress category.

2.2.3 RAS frequency Assessment

This part included seven questions evaluating the symptoms, frequency, and severity of RAS in order to determine the RAS rate within the Armenian ethnicity as well as the treatment measurements taken by these individuals, the questions and options are listed below:

Table 4: RAS related questions and options.

Questions	Options
Did you experience any type of mouth ulcer before?	• Yes • No
When was the last time you experienced an ulcer ?	• Currently have it • One month ago • 6 months ago • One year ago or more
Frequency of ulcer?	• Once • 2-3 • 4 or more • None
How long do they last ? (Days)	• 0-2 • 3-5 • 6 or more
How painful is it ?	• No pain

	• Slight pain • Moderate pain • Severe pain
Any treatment taken ?	• No treatment • Topical gel or vitamins • Home remedies
Do you regulary eat food that is	• Salty • Acidic • Spicy • None

Chapter Three: Results and Discussion

3.1 Results

The questionnaire was found to be reliable; Cronbach's alpha coefficients for all variables were .715 consider it to have satisfactory internal consistency.

A total of 97 answers were collected, with female participants accounting for 78.4% of the total and male participants accounting for 21.6%. The majority are in their twenties and thirties. 55.7% were university students or graduates, while 24.7% had postgraduate degrees, and 19.6% were high school students or lower. Table 6 summarized the demographic characteristics of the participants.

Table 6: Demographic Characteristics of Participants

Variable	Category	N (%)
Gender	Male	21 (21.6)
	Female	76(78.4)
Age	< 20	5(5.2)
	20 – 40	77(79.4)
	> 40	15(15.5)
Education	University student or graduate	54(55.7)
	Postgraduate (Master's, PhD.)	24(24.7)
	High school or less	19(19.6)
Income	Low	16(16.5)
	Enough	70(72.2)

		Enough and more	11(11.3)

Recurrent Aphthous Stomatitis Questionnaire and response:

The main finding of the study was that 70% of the participants had an ulcer six months to a year ago. 52.6% have only one frequency, 53.6% have them for 3-5 days, and 73.2% have minor or no pain. Table 7 summarized the responses to the recurrent Aphthous stomatitis questionnaire.

Table 5: RAS history Questionnaire and responses

	Questions	**Answers**	**N (%)**
1.	**Did You Experience Any Type of Mouth Ulcer Before?**	Yes	80 (82.5)
		No	17(17.5)
2.	**When Was the Last Time You Experienced an Ulcer**	Currently Have It	5 (5.2)
		1 Month Ago	23(23.7)
		6 Months Ago	17(17.5)
		1 Year Ago, & More	52(53.6)
3.	**Frequency Of Ulcer?**	None	17(17.5)
		Once	51(52.6)
		2-3	23(23.7)
		4 And more	6(6.2)
4.		0-2	31(32)

		3-5	52(53.6)
	How Long Do They Last? (Days)	6 And more	14(14.4)
5.	**How Painful Is It?**	No Pain	31(32)
		Slight Pain	40(41.2)
		Moderate Pain	24(24.7)
		Sever Pain	2(2.1)
6.	**Any Treatment Taken?**	No Treatment	66(68)
		Topical Gel or Vitamins	19(19.6)
		Home Remedies	12(12.4)
7.	**Do You Regularly Eat Food That Is**	None	24(24.7)
		Salty	21(21.6)
		Acidic	7(7.2)
		Spicy	17(17.5)
		Acidic and salty	20(20.6)
		spicy and salty	8(8.2)
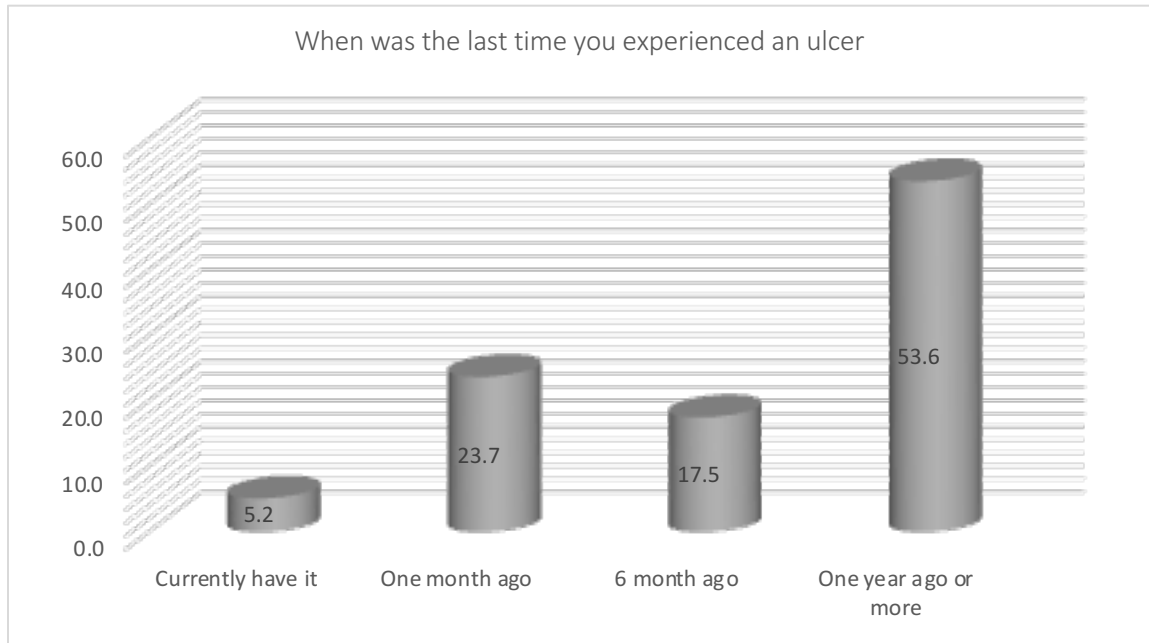
Figure 4: Participants' experience with an ulcer.

The Perceived Stress Scale (PSS) Questionnaire and response:

Evaluation Results of the Perceived Stress Scale (PSS): The stress levels of the 97 participants in the survey showed that the majority (83.5%) had a low to moderate stress level, which was distributed as low, moderate, or high, accounting for 24.74%, 58.76%, and 16.49%, respectively. Figure 6, Table 8 summarized the responses to the perceived stress scale (PSS) questionnaire.
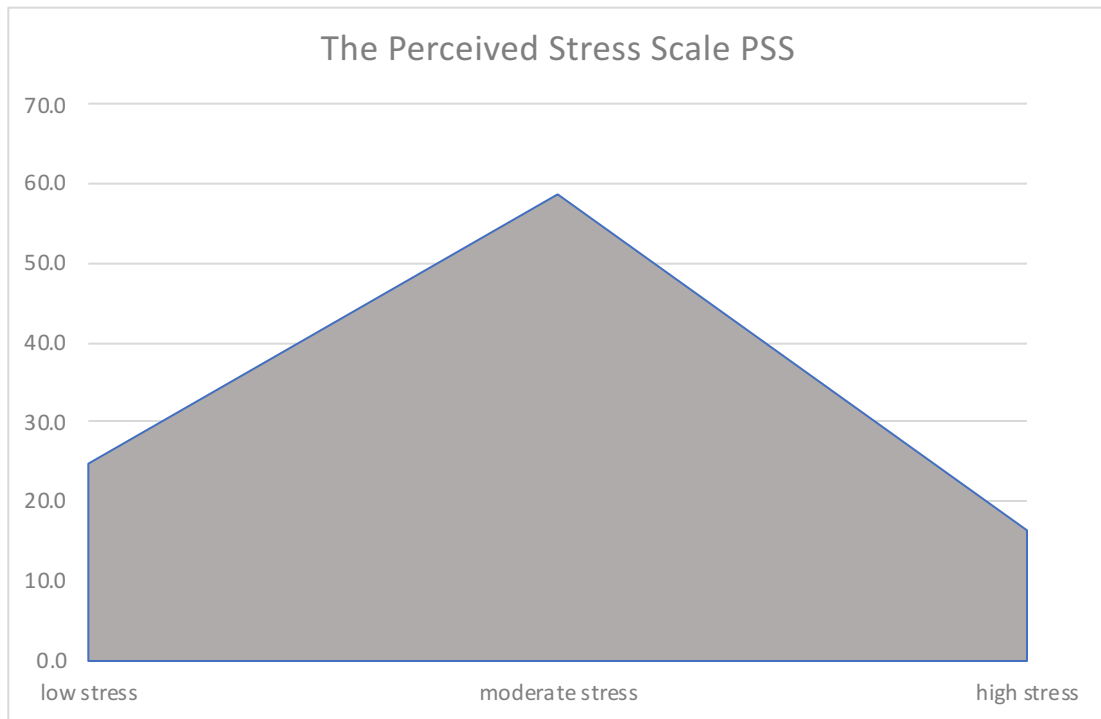
Figure 5: The Perceived Stress Scale PSS average.

Table 6: The Perceived Stress Scale (PSS) Questionnaire and responses.

	Questions	Answers	N (%)	Mean	SD	P value
1.	In the last month, how often have you been upset because of something that happened unexpectedly?	Negative	26 (26.8)	2.23	1.195	0.00
		Neutral	33 (34)			
		Positive	38 (39.2)			
2.	In the last month, how often have you felt that you were unable to control	Negative	47 (48.5)	1.76	1.305	0.00
		Neutral	21(21.6)			

	the important things in your life?	Positive	29 (29.9)			
3.	In the last month, how often have you felt nervous and stressed?	Negative	27 (27.9)	2.27	1.159	0.00
		Neutral	30 (30.9)			
		Positive	40 (41.3)			
4.	In the last month, how often have you felt confident about your ability to handle your personal problems?	Negative	52 (53.6)	1.53	0.914	0.00
		Neutral	28 (28.9)			
		Positive	17 (17.5)			
5.	In the last month, how often have you felt that things were going your way?	Negative	32(33)	1.98	1.041	0.00
		Neutral	38 (39.2)			
		Positive	27(27.9)			
6.	In the last month, how often have you found that you could not cope with all the things that you had to do?	Negative	45(46.4)	1.64	0.959	0.00
		Neutral	35 (36.1)			
		Positive	17 (17.5)			
7.	In the last month, how often have you been able to control irritations in your life?	Negative	39 (40.2)	1.80	1.037	0.00
		Neutral	35 (36.1)			
		Positive	23 (23.7)			

8.	**In the last month, how often have you felt that you were on top of things?**	Negative	32 (33)	1.97	1.025	0.00
		Neutral	35 (36.1)			
		Positive	30 (30.9)			
9.	**In the last month, how often have you been angered because of things that happened that were outside of your control?**	Negative	43 (44.3)	1.85	1.083	0.00
		Neutral	27 (27.8)			
		Positive	27 (27.8)			
10.	**In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?**	Negative	48 (49.5)	1.64	1.165	0.00
		Neutral	26(26.8)			
		Positive	23 (23.7)			

Caffeine Consumption Questionnaire and response:

The results of this section of the questionnaire revealed that 69.2% of respondents consume two or more sources of caffeine, 49.5% consume it three or more times per day, and 51.5% began using caffeine before the age of eighteen. Furthermore, when asked how caffeine makes them feel, 51.5% said relaxed, calmed down, and improved their mood, 38.1% said alert, energized, and less sleepy, and 10.3% said it improved their mood, helped them think clearly, pay more attention, and sharpen the memory (See Table 9 and Figure 7).

Table 7: Caffeine Consumption Questionnaire and responses.

	Questions	Answers	N (%)
1.	**Sources of caffeine**	Coffee	18(18.5)
		Tea	8(8.2)
		Chocolate	3 (3.1)
		Energy Drinks	1 (1)
		two types	35(36.1)
		more than two types	32 (33.1)
2.	**How often do you consume Caffeinated beverages?**	Once a day	28(28.9)
		2-3 times daily	43(44.3)
		4-5 times daily	5(5.2)
		More than 5 daily	2(2.1)
		1-3 times weekly	9 (9.3)
		4-6 times weekly	10 (10.3)
3.	**What time of the day do you usually consume caffeine?**	Morning	28 (28.9)
		Afternoon	19 (19.6)
		Evening	15 (15.5)

|		Morning and Evening	12 (12.4)
		Morning and Afternoon	23 (23.7)
4.	**When do you prefer to have caffeine?**	Before eating	23 (23.7)
		While eating	16 (16.5)
		After eating	58 (59.8)
5.	**At what age did you start taking caffeine?**	8 – 12	8 (8.2)
		13 – 17	46 (47.4)
		Over 18	39 (40.2)
		Under 8	4 (4.1)
6.	**Caffeine makes me feel**	Relax, calm down, and improves my mood	50 (51.5)
		Alert, energized and less sleepy	37 (38.1)
		Think clearly, pay more attention and sharpen my memory	10 (10.3)
7.	**Cup Size**	240 ml	41 (42.3)
		360 ml	39 (40.2)
		480 ml	16 (16.5)
		600 ml	1 (1)
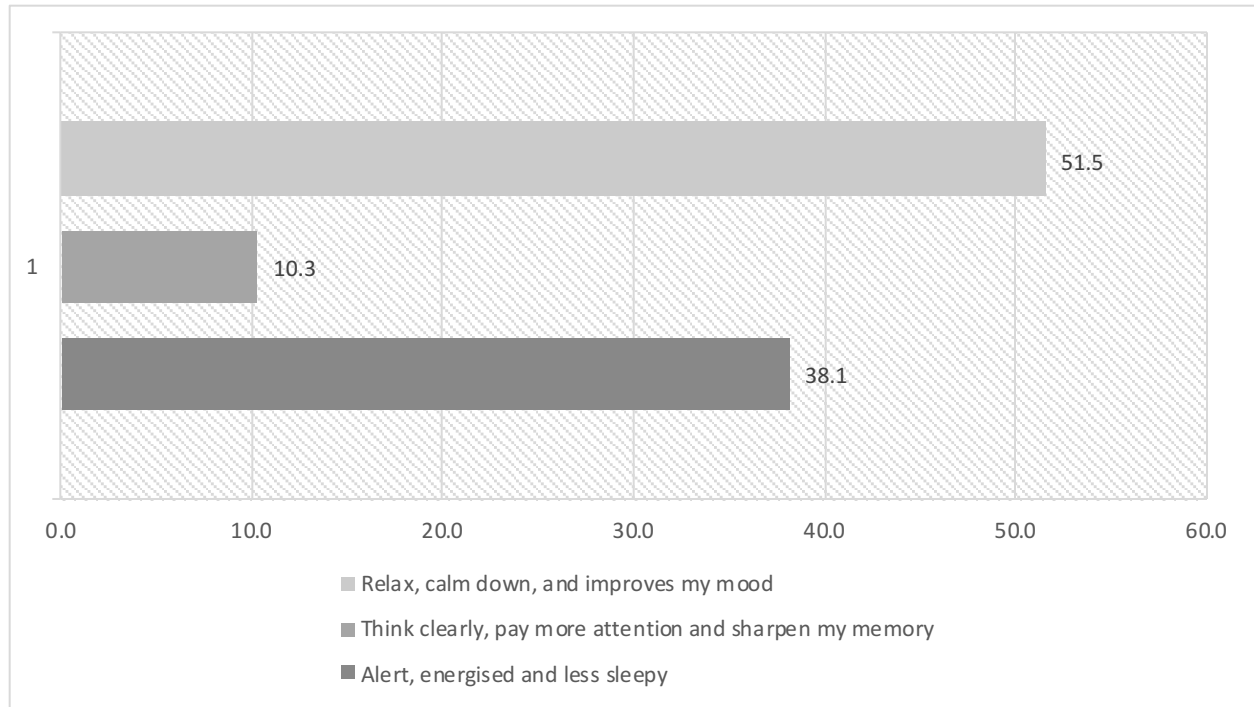
Figure 7: How caffeine made the participants feel.

Correlation between Variable:

Kendall's tau-b (τb) correlation coefficient was applied to find the strength and direction of association that exists between the two variables, the number of caffeinated drinks taken per day in ml and the occurrence of Aphthous stomatitis. A p value $p<0.05$ was considered statistically significant (two-tailed). (See Table 10).

Table 10: Kendall's tau correlation

Correlations			Did you experience any type of mouth ulcer before?	Amount of caffeine taken per day in ml
Kendall's tau_b	Did you experience any type of mouth ulcer before?	Correlation Coefficient	1.000	-.240*
		Sig. (2-tailed)	.	.015
		N	97	97
	Amount of caffeine taken per day in ml	Correlation Coefficient	-.240*	1.000
		Sig. (2-tailed)	.015	.
		N	97	97
*. Correlation is significant at the 0.05 level (2-tailed).				

The result interpreted as follows: a correlation value of -.240* between two variables would indicate that a significant and negative relationship exists between the two. A negative correlation indicates that as the rank of one variable increases, the other one decreases.

3.2 Discussion

The impact of oral lesions such as RAS on the quality of life is considered very important as these lesions effect speech, nutrition and general comfort, and the incidence of this condition is found to be higher in individuals who experience high levels of stress **(Thevara et al, 2020).** Caffeine being the most widely consumed psychostimulant substance has been reported to have beneficial effects on certain neurotransmitters such as dopamine and serotonin which in turn lead to mood improvements and feeling of wellbeing **(Singh, 2016).**

In this study 97 questionnaires were collected from individuals with Armenian ethnicity exploring their caffeine habits, stress levels and RAS history.

And according to our data it's been found that 83.5% of the participants had low-to-moderate stress levels and that 44.3% of the participants consume caffeine two to three times per day, these findings correlate with the a previous study done by **Haskell-Ramsay *et al*, (2018)** where they did a double-blind test to compare the effects of regular coffee, decaffeinated coffee and placebo, it was found that regular coffee had overall a higher mood improvement and decreased mental fatigue.

Similar results were reported in previous literature such as a study published in the journal of psychopharmacology that noticed a reduction in stress levels in both men and women that consume caffeine **(Smith, 2002).**

Our date also found that 53.6% of the participants haven't had RAS in the last 12 months, this may be associated to the low-to-moderate stress levels which agrees with the results of the study done by **Albanidou-Farmaki *et al*, (2008)** who found a positive association between RAS and the participant's anxiety levels.

Many studies suggested that psychological disturbances such as stress and anxiety could play a role in the etiology of RAS lesions. However, some studies have shown variable results like the study done by **Pavle Picek** *et al,* **(2012**) who concluded that psychological disturbances didn't precede the development of RAS in the subjects.

Due to the vague etiology, even in the present-day research and studies on RAS continues to be noteworthy in order to be able to understand the condition more.

3.1   Conclusion

The data we collected in our research found that the prevalence of RAS in the Armenian ethnicity was infrequent, over half of the participants hadn't gotten RAS in the past 12 months. Which may correlate to their caffeine consumption habits that can lead to stress reduction. However, caution should be taken when consuming caffeine as harmful effects are potential with large doses.

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