

# Relationship Between the Surgical Difficulty of Mandibular Third Molar Extraction Under Local Anesthesia and the Postoperative Level of Salivary Alpha Amylase

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## ABSTRACT

**Objective:** To assess the relationship between the surgical difficulty of lower third molar removal using time and technique, and the postoperative salivary amylase concentration.

**Methods:** patients submitted for surgical removal of impacted lower wisdom teeth for any indication were included in this prospective cohort study. Three samples of saliva were taken from each patient (preoperative, 48 hours postoperative, and 7 days postoperatively) and the concentration of salivary amylase is measured by Enzyme Linked Immunosorbent Assay (ELISA) kit. The surgical difficulty was assessed using time of operation and the technique for extraction and correlated with the postoperative level of salivary amylase to test if there is any relationship between them, and correlated with other variables including Age, gender, and classification of the impacted lower wisdom tooth (Winter's and Pell and Gregory's).

**Results:** 34 patients participated in this study. 15 [44.12%] males and 19 [55.88%] females; 36 impacted teeth were removed (19 in right side [53%], and 17 in the left side [53%]). The mean  $\pm$  SD of preoperative, 48 hours and 7 days postoperative salivary amylase concentration were  $126.2 \pm 31.97$ ,  $131.8 \pm 48.99$ , and  $127.0 \pm 32.09$  respectively. 17 impacted teeth were mesioangular, 11 were vertical and 8 were horizontal according to Winter. The surgical difficulty according to time was low in 7, moderate in 14, and high in 15 cases. While difficulty according to technique was low in 5, moderate in 11, and high in 20 cases. The correlation of amylase concentration with the surgical difficulty was ( $r = 0.23$ ,  $P = 0.176$ ).

**Conclusions:** there was non-significant correlation between the surgical difficulty (measured by time and technique) and the change in salivary amylase concentration.

## INTRODUCTION

The use of saliva as a diagnostic fluid is gaining popularity since saliva sample collection is a straightforward, non-invasive procedure. Saliva samples are safe for the health care provider and the patient, as well as simple and cost-effective storage. These qualities enable the monitoring of many biomarkers in youngsters, the old age, and people who refuse to cooperate in the collection of urine or blood samples. The use of saliva as a diagnostic fluid is a good option not only for the aforementioned reasons, but also for the fact that the essential biochemical characteristics in blood and saliva have a direct relationship.<sup>1</sup>

The three pairs of the major salivary glands (parotid, submandibular, and sublingual glands) create the majority of saliva (90 percent), while the minor salivary glands (located in the labial, buccal, lingual, and palatal portions of the oral mucosa) produce a tiny amount (10 percent)<sup>2,3</sup>. Even though saliva is 99 percent water, it contains a variety of chemicals that have been dispersed from blood via paracellular or transcellular pathways<sup>3,5</sup>. Saliva serves a variety of purposes, including digestion (by lubricating and binding the alimentary bolus and initiating starch digestion), gustatory experience (by solubilizing dry food), protection (by mechanically mobilizing alimentary detritus), and antimicrobial action (lysis of the bacterial cell wall due to lysozyme).<sup>4</sup>

Saliva, along with gingival crevicular fluid (GCF), have been used medication monitoring and to diagnose numerous oral and systemic disorders, according to a considerable existing literature.<sup>6</sup>

Saliva-based tests have been successfully employed in HIV infection diagnosis<sup>7</sup>, renal disease monitoring<sup>8</sup>, cardiometabolic risk prevention<sup>9</sup>, viral nucleic acid detection and quantification<sup>10</sup>, forensic medicine investigations<sup>11</sup>, dental research<sup>12,13</sup>, and drug misuse monitoring<sup>11</sup>. There have also been some studies that suggest using saliva to monitor physically active people, incremental effort tests<sup>14-16</sup>, and psychological stress.<sup>17</sup>

The surgical extraction of lower third molars is among the commonest procedures done in oral surgery clinics, and it is frequently linked with postoperative pain, facial swelling, and trismus, all of which can result in jaw function loss. Many complex elements play a role in these scenarios, but the majority of them stem from inflammation triggered by surgical damage.<sup>18,19</sup>

Saliva, crevicular fluid, and serum are the most common fluids utilized in inflammation prognostic tests. Inflammatory salivary indicators such as cytokines (TNF- and IFN-, IL-1,4,6,8,10), IgA,  $\alpha$ -amylase, cortisol, and total proteins have been widely used in biomedical prognostication in recent decades.<sup>20-24</sup>

Salivary alpha amylase (SAA) is one of the most plentiful components in saliva, constituting 10% – 20% of the total salivary protein content<sup>25</sup>. The highly differentiated epithelial acinar cells of the exocrine salivary glands, primarily the parotid glands, manufacture it locally<sup>26</sup>. SAA function in digestion by hydrolyzing  $\alpha$ -1,4 glycosidic linkages in polysaccharides converting it into simpler sugars<sup>27</sup>. Furthermore, alpha amylase has been proposed as a means of inhibiting bacterial affinity to oral surfaces and facilitating bacterial removal in the mouth.<sup>28</sup>

The SAA has been proposed as a salivary marker for stress-related physiological alterations.<sup>17,29</sup>

The autonomic nervous system (ANS) activates the hypothalamus-pituitary-adrenal (HPA) axis in response to stress and inflammation, and noradrenergic neurons synthesis and release catecholamines such epinephrine, norepinephrine, and dopamine; SAA from acinar cells of the salivary glands, which are supplied by sympathetic and parasympathetic branches of the ANS, can be found in saliva, while these biomarkers are tested in blood. As a result, SAA concentration has been utilized as a dependable stress marker in recent years.<sup>30,31</sup>

Several research have found that SAA rises after surgical excision of impacted lower wisdom teeth<sup>32</sup>, but none have looked into the link between rising SAA and surgical difficulty.

The goal of this research is to see if there is a link between increasing SAA level and surgical difficulty (surgical time and technique).

## METHODS

This prospective cohort study was carried out in the department of oral and maxillofacial surgery, College of Dentistry, University of Baghdad for the duration of six months (January 2022 to June 2022).

**The inclusion criteria were:**

- Healthy subjects submitted for surgical removal of impacted lower wisdom teeth for any indication.

- o Age: 18 years and older.
- o Gender: both genders.

**Exclusion criteria were:**

- o Medically compromised subjects.
- o History of irradiation of the head and neck.
- o Pregnant women.
- o Smokers and/or alcoholics.
- o Impacted lower wisdom teeth presented with any pathology such as cyst or tumor.

The study included patients meeting the aforementioned criteria. At the first visit the medical and dental history were documented, clinical examination extra orally and intraorally performed and recorded any abnormalities. Investigations included orthopantomogram and CBCT if needed. The study procedures and objectives were explained to all of the patients, and after answering any inquiries from them, they were instructed to sign informed consent for participating in the study.

Ethical approval was confirmed by the University of Baghdad's College of Dentistry's Research ethics committee (project no.395121).

Afterward, the patients were prepared for the surgery, and before administering local anesthesia, the patients were instructed to sit in upright position in the dental chair with their heads tilted forward and to stop swallowing saliva to allow it to accumulate in the floor of the mouth; then the patients spit the accumulated saliva into sterile container (passive spitting method). The obtained saliva samples were transferred into Eppendorf tube and stored at -20 °C until tested.

2% xylocaine (lidocaine HCL) was given to the patients as inferior alveolar nerve block and infiltration for the long buccal nerve. After the patient recorded anesthesia, the surgical procedure began with an envelope flap reflection, followed by bone removal and/or tooth sectioning if needed, then application of elevator for tooth delivery.

Cleaning of the socket is achieved by copious saline irrigation, and sharp bone trimming by bone file. Then suturing using 3-0 silk suture, and instructions were given to the patients to

bite on the gauze for about 30 minutes and avoid rinsing their mouth in the first 24 hours. The postoperative sequels were explained to the patients and prescribed Amoxil and flagyl (500mg three times daily), along with paracetamol 1g for pain relief.

The surgical difficulty was estimated by time and technique. The time of surgery is recorder by digital stopwatch from the beginning of incision to the last stich, so the surgical difficulty is classified into low (less than 15 minutes), moderate (15 to 30 minutes) and high (longer than 30 minutes). The surgical technique for the extraction was categorized into low (only use of elevator), moderate (bone removal and elevator), and high (bone removal, tooth sectioning and elevator).

Forty-eight hours postop. A follow up visit was conducted and another sample of saliva was obtained; Seven days postoperatively the sutures were removed and another sample was obtained and stored as explained earlier.

The concentration of SAA was measured using Enzyme Linked Immunosorbent Assay (ELISA) kit of (Demeditec Diagnostics GmbH, Germany).

GraphPad prism windows software (GraphPad software, La Jolla, CA, USA) version 9 was used for statistical analysis.

**RESULTS**

34 patients participated in this study. 15 [44.12%] males and 19 [55.88%] females; 36 impacted teeth were removed, 19 in right side [53%], and 17 in the left side [53%]. The mean ± SD of preoperative, (48 hours and 7 days) postoperative salivary amylase concentration were 126.2±31.97, 131.8±48.99, and 127.0±32.09 respectively. 17 impacted teeth were mesioangular [47.22%], 11 were vertical [30.56%], and 8 [22.22%] were horizontal according to Winter. The surgical difficulty according to time was low in 7, moderate in 14, and high in 15 cases. While difficulty according to technique was low in 5, moderate in 11, and high in 20 cases

Descriptive statistics, and the difference between pre- and post-operative SAA concentration are summarized in table 1.1, other variables are summarized in table 1.2.

Table 1: descriptive statics of SAA concentration.

	SAA concentration (U/ml)			P value
	preoperative	48 hours postoperative	7 days postoperative	
Mean	126.2	131.8	127.0	0.636 [NS] a
Standard deviation (SD)	31.97	48.99	32.09	
Minimum	69.80	56.20	70.90	
Maximum	198.2	271.3	199.4	
Range	128.4	215.1	128.5	

A: Repeated measures ANOVA. NS: non-significant.

Table 2: Relation of different variables with SAA concentration.

Variables	SAA level U/ml			P value
	preoperative	48 hrs. postoperative	7 days postoperative	
<b>Winter's classification</b>				
Mesioangular	131.7 ± 33	136 ± 55.82	132.7 ± 33	0.5301 a [NS]
Vertical	128.7 ± 33.15	131.7 ± 43.5	127.5 ± 33.71	
Horizontal	118 ± 29.3	122.8 ± 45.18	118.6 ± 29.39	
<b>Pell &amp; Gregory classification</b>				
Position A	124.4 ±33.69	130.3 ± 67.44	125.2 ± 33.81	0.646 b [NS]
Position B	127.5 ±31.46	132.8 ± 31.85	128.2 ± 31.59	
Class I	124.7 ±36.25	132.3 ± 33.41	125.5 ± 36.32	
Class II	127 ± 30.12	131.5 ± 56.65	127.8 ± 30.27	b [NS]
<b>Surgical time</b>				
Low	127.1 ±19.39	134.9 ± 37.85	128 ± 19.27	0.377 c [NS]
Moderate	129.7 ±35.53	134.4 ± 41.21	130.6 ± 35.64	
High	122.6 ±34.56	127.9 ± 61.5	123.6 ± 34.53	
<b>Surgical technique</b>				
Low	126.4 ±32.31	131.8 ± 57.34	126.4 ± 32.77	0.365 a [NS]
Moderate	125.1 ±31.82	129.6 ± 29.49	126 ± 32	
High	126.7 ± 33.6	133 ± 57.16	127.6 ± 33.62	

- A Kruskal-Wallis test.
- B Mann Whitney test.
- C one way ANOVA.
- NS non-significant.

Correlation between the change in the level of SAA and the duration of surgery in this study was non-significant ( $r = -0.23$ ,  $p = 0.176$ ).

## DISCUSSION

Dental treatment and other events produce anxiety, biomarkers in saliva have been suggested as a tool for monitoring anxiety<sup>33</sup>. The use of SAA biomarker in this study was decided on previous studies that shown significant increase in SAA in stressed individuals<sup>17,34</sup>, and dental treatment or surgery also result in significant increase in SAA levels<sup>36,32</sup>. Our study showed the contrary, SAA increased but non-significantly following mandibular third molar surgery; this can be explained by timing of saliva samples; Gutiérrez-Corrales et al. took samples 1 and 2 hours postoperatively which shown significant increase<sup>32</sup>, while we took samples 48 hours and 7 days postoperatively which shown non-significant increase in SAA concentration.

Pearson correlation in our study showed non-significant association between the time of surgery and SAA concentration.

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