ISSN: 0067-2904

The effect of smoking on serum and saliva AMP-aminohydrolase (AMPDA) and adenosine aminohydrolase (ADA) activities

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Received: 23/3/2024 Accepted: 1/9/2024 Published: 30/9/2025

Abstract

Adenosine deaminase (ADA), also referred to as adenosine aminohydrolase, is a key enzyme that plays a crucial role in purine metabolism. AMP-aminohydrolase, also known as AMP deaminase (AMPDA), facilitates the conversion of adenosine monophosphate (AMP) into inosine monophosphate (IMP). Cigarettes primarily consist of various substances. This study aimed to investigate the influence of cigarettes smoking on serum and saliva ADA and AMPDA activities, exploring the diagnostic potential of these enzymes in smoking- related diseases. Two hundred twenty subjects were enrolled, including 100 healthy smokers and 120 healthy non-smokers with measurement being made of serum and saliva ADA and AMPDA activities as well as complete blood count (CBC). There were highly significant increase in saliva ADA and AMPDA activities in smokers' group in comparison to non-smokers as well as significant increase in serum ADA and AMPDA activities of smokers' group in comparison to non-smokers group. The current study indicates that serum and saliva ADA and AMPDA levels increase in smokers due to effect of cigarettes smoking. The findings strongly suggest the potential of saliva AMPDA as a diagnostic marker for tongue cancer, as well as the role of ADA in diagnosing lung disease and coronary artery disease associated with prolonged smoking.

Keywords: Adenosine Aminohydrolase; AMP-aminohydrolase; Coronary Artery Disease; tongue Cancer; Saliva; Smoking

تأثير التدخين على نشاطات إنزيم AMP–أمينوهيدروليز (AMPDA) وإنزيم الأدينوسين أمينوهيدروليز (ADA) في السيرم واللعاب

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الخلاصة

اديموسين ديأمينيز (ADA), الذي يُعرف أيضاً بأسم الاديموسين امينوهيدروليز ,هو انزيم رئيسي يلعب دوراً حاسماً في ايض البيورين. انزيمAMP –أمينوهيدروليز المعروف ايضا بأسAMP – ديأمينيز (AMPDA), يسهل تحويل الاديموسين احادي الفوسفات الى انيوسين احادي الفوسفات. تتكون السجائر بشكل أساسي من مواد مختلفة. تهدف هذه الدراسة الى التحقيق في تأثير تدخين السجائر على فعاليات ADA و AMPDA في المصل واللعاب, واستكشاف الأمكانيات التشخيصية لهذه الانزيمات في الامراض المرتبطة بالتدخين. تم تسجيل مئتين وعشرون مشتركاً, بما في ذلك 110 مدخناً سليماً و 120 غير مدخن سليم , حيث تم قياس فعالية الانزيم في المصل و اللعاب بالاضافة الى صورة الدم الكاملةCBC . لوحظ زيادة معنوية عالية في فعاليات ADAو AMPDA في اللعاب لدى مجموعة المدخنين مقارنة مع مجموعة غير المدخنين, وكذلك زيادة معنوية في فعاليات ADA و AMPDA في المصل لدى مجموعة المدخنين مقارنة مع مجموعة غير المدخنين. تشير الدراسة الحالية الى مستويات ADAو AMPDA في المصل واللعاب تزداد لدى المدخنين بسبب تأثير تدخين السجائر. تشير النتائج بقوة على إمكانية استخدام AMPDA في اللعاب كعلامة تشخيصية لسرطان اللسان, وكذلك دور ADA في تشخيص أمراض الرئة ومرض الشرايان التاجي المرتبطة بالتدخين المطول.

1. Introduction

Cigarette smoke is harmful due to its numerous toxic compounds, which induce oxidative damage and trigger the clumping of platelets [1]. One of the main culprits is nicotine, a primary alkaloid in tobacco and one of the over 4,000 substances present in cigarette smoke. In addition to cigarette, nicotine exposure is obtained through preparations for quitting smoking using nicotine replacement therapy [2]. Nicotine readily passes through the blood-brain barrier and is highly soluble in lipids [3]. It increases the level of oxidative stress and produces free radicals. It increases the flow of free fatty acids to the liver by stimulating lipolysis, and increased VLDL production that results from the liver's re-esterification of free fatty acids explains smoking's atherogenic effects [4]. Although it has been linked to lower body weight and food consumption, there is conflicting evidence about its efficacy as a medication for weight loss. After extended exposure, nicotine has been observed to diminish insulin sensitivity [5]. Moreover, nicotine has been linked to liver disease, renal injury due to ischemia-reperfusion [6], ulcerative colitis [7], sepsis [8], and type 1 and the type 2 diabetes mellitus [9].

Adenosine deaminase; (ADA) is one common enzyme in mammalian that involved in metabolism of purine base. By means of an irreversible deamination reaction, it converts adenosine into inosine, so clearing it from the cellular environment. Moreover, ADA has been shown to activate the immune system in several tissues [10, 11], with skeletal muscle, liver, adipose, and lymphoid tissues exhibiting comparatively high levels of ADA activity. Similarly, it had been noted that diabetic mellitus type 2 patients exhibited enhanced ADA activity [12]. The combined activity of ADA's two isoenzyme expressed as total ADA activity. In illnesses where the number of T lymphocytes rises, it may lead to serum ADA elevate [13-16]. This is why it has been employed as marker for chronic inflammation [13] and cell-mediated immunity [17]. Human serum adenosine deaminase activity has been found to be elevated in number of diseases, including immune-mediated conditions like chronic lymphocytic leukemia [18], breast carcinoma [19], or bladder carcinoma [20], and inflammatory condition like chronic tonsillitis, rhinosinusitis, or otitis media [21].

An enzyme known as Adenosine monophosphate (AMP) deaminase (AMPDA; EC 3.5.4.6), composed of four subunits, facilitates the hydrolysis of an amino group from 6-position of the adenine nucleotide ring. It is situated at pivotal juncture in adenylate catabolic pathway, AMPDA is highly regulated and diversified enzyme [22]. Purine nucleotide breakdown mostly occurs in the liver. AMP deaminase (AMPDA) converts 5`-adenosine monophosphate (AMP) into 5`-inosine monophosphate (IMP) [23]. It exists in all vertebrate cell types and has a significant physiological and biochemical role related to the metabolism of nucleotides and energy. The processes involving adenylosuccinate synthetase and adenylosuccinate lyase convert 5`-adenosine monophosphate to inosine 5`-monophosphate (IMP). Subsequently, IMP can undergo further breakdown to inosine or entering cycle of purine nucleotide [24]. AMPDA initiates this process through its catalytic action. By facilitating the conversion of the substantial adenylate pool into guanine nucleotides, AMPDA effectively integrates the metabolic pathways of adenine and guanine [25]. Uric acid (UA) serves as the ultimate byproduct of 5`-adenosine monophosphate metabolism through AMPDA in humans, constituting a component to of the purine degradation pathway. Despite the antioxidant qualities of UA, elevated uric acid levels have been linked to mitochondrial dysfunction, oxidative stress, inflammation, the intensity of kidney disease and the presence sarcopenia [26, 27]

Saliva is an organic fluid that may be used to identify biomarkers for systemic disorders as well as oral diseases [28]. Increased level of ADA activity in saliva has been found to be a reliable indicator in persons with both systemic diseases like obesity [21] and local pathologies like Sjogren's syndrome [14] and oral cancers [18 ,20].

The study aimed to explore potential distinctions in serum and saliva ADA and AMPDA activities between smokers and non-smoker subjects.

2. Methods

Blood samples were collected from 100 individuals who smoke cigarettes and 120 non-smokers (control group) with ages ranging from 18 to 45 years from staff and students at University of Technology-Iraq, within four months. A review of the participants' medical histories revealed no instances of chronic diseases among them. Venous blood, approximately 10 mL, was drown using a disposable syringe. Subsequently, 2 mL of the blood was placed into EDTA tube and gently mixed to prevent clotting for determination of complete blood count (CBC) directly by automatic hematological analyzer (Advia, Siemens, Germany). Ten to fifteen minutes were given to the residual blood to coagulate at room temperature, following by centrifugation at 1500 x g for ten minutes to separate serum [30]. The serum was frozen in a new tube at (-20^{o} C).

Unstimulated saliva sampling were collected in morning (fasting, no smoking or brushing of the teeth), after participants thoroughly rinsed their mouths with distilled water, they were instructed to spit back into a clean sterile container and centrifuged for 15 min at 1100 x g and frozen at (-20^{o} C) [31].

The ADA activity was determined following the Giusti method [32], measured with a spectrophotometer (UV-1100, England), at 630nm by direct detection of ammonia that formed by enzymatic conversion of adenosine to inosine and ammonia, and the unit of ADA is defined as the amount of enzyme forming one µmole of ammonia in 1 min. AMPDA activity was estimated using the Gromashevskaia method that depends on measuring the concentration of IMP via spectrophotometer [33]. All statistical analyses in this study were

performed using statistical Package for Social Science (SPSS) version 29.0 windows (statistical Package for Social Science, Inc., Chicago, IL USA). Expressive analysis was presented as the mean ± standard deviation of the variable. The significance of difference between mean values was assessed using the Student's t-Test, when $p < 0.05$ was considered significant, $p > 0.05$ deemed non-significant. Pearson correlation analysis was used to test the liner relationship between parameters.

3. Results and Discussion

The study consists of 100 cigarette smokers in good health and 120 healthy non-smokers as control group. Table 1 displays the mean (± SD) of age for both control and smokers groups, indicating a successful matching with no significantly different between two groups of the current study. The result in Table 1 shows the comparison of mean (± SD) of CBC between smokers and non-smokers. A noteworthy decrease ($p<0.01$) in platelet count level was observed in smokers compared to non- smokers. Additionally, the present study shows a significant elevated level of MCV in smokers group compared to non-smokers group.

Table1: The mean (± SD) of age and CBC for smokers and non-smokers

Characteristic	Cigarette smokers[n=100] [Mean ± SD]	Non-smokers [n=120] [Mean ± SD]
Age [year]	35.55±5.75	36.50± 5.80
RBC x10^6 cell/ml	5.50±0.44	5.80± 0.45
Hb [g/dl]	15.99±0.38	14.00±0.88
HCT[%]	48.75±1.85	42.51±2.10
MCV [fL]	91.66±2.25 [a]	86.00±3.55
MCH [pg/cell]	32.50±1.50	29.55±1.33
MCHC [g/dl]	36.75±0.76	36.00±0.65
RDW [%]	13.77±0.55	12.95±0.62
Platelets x10^3 cell/ml	260.99±45.55 [a]	298.00±44.75
MPV[fL]	8.88±0.70	8.15±0.75

[a] *mean significant when* compared with control group ($p<0.01$).

The mean levels of serum ADA and AMPA showed a significant increase ($p<0.01$) in smokers group when compared to non-smokers group and highly significant increases ($p<0.001$) in level of saliva ADA and AMPA in smokers group when compared to non-smokers, as shown in Table 2.

Table2: The mean (± SD) of ADA and AMPDA activities in serum and saliva for smokers and non-smokers groups.

Parameters	Cigarette smokers [n=100] [Mean ± SD]	Non-smokers [n=120] [Mean ± SD]	*P* Value
Serum ADA (U/L)	28.90 ±0.50	12.9 ±0.40	0.01
Serum AMPDA (U/L)	30.99±0.40	13.50±0.55	0.01
Saliva ADA (U/L)	90.75±1.45	40.55±2.25	0.001
Saliva AMPDA (U/L)	164.66±1.95	80.50±1.99	0.001

A correlation analysis was conducted between saliva AMPDA and other parameters. The results revealed a positive correlation with saliva ADA ($r=0.456$, $p<0.01$) and serum ADA ($r=0.220$, $p=0.02$). Whereas, no significant correlations were found with other parameters:

serum such as AMPDA (r=0.1, p=0.9), Hb (r=0.43, p=0.67), MCV (r=0.145, p=0.1), MCH (r=0.41, p=0.6), RBC (r=-0.8, p=0.4) and platelet (r=-0.045, p=0.65). Correlation analysis between serum AMPDA and other parameters revealed the presence of medium positive correlation with serum ADA (r=0.409, p<0.01) while non-significant correlations with saliva ADA (r=-0.096, p=0.3), RBC (r=-0.041, p=0.68), MCHC (r=-0.16, p=0.1) and platelet (r=0.005, p=0.9) were found.
Also, correlation analyses were performed between serum ADA with saliva ADA (r=0.099, p=0.32), MCV(r=0.111, p=0.27), RBC (r=-0.68, p=0.49), Hb (r= -0.78, p=0.43) and platelet (r=-0.41, p= 0.68).

The ADA enzyme is typically found in human tissue such the spleen and thymus, with the gastrointestinal system having the greatest concentration and brain having modest activity [34]. In an atherosclerosis mouse model, it has been reported that rising ADA activity preceded macrophage accumulation and vascular lipid accumulation, so it increases with the development greater plague thereafter [35]. Increased vascular ADA activity has been suggested to serve as an early warning sign and potential catalyst for the progression of atherosclerosis. The enzyme has been demonstrated to be essential for the development of T and B lymphocytes as well as for the differentiation of monocytes into macrophages, and it has a major impact in detoxification processes [14]. The frequency of chronic obstructive pulmonary disease (COPD) is most often linked to tobacco use [36]. By attracting different inflammatory cells that release inflammatory mediators, smoke causes an inflammatory reaction in lung airways [37]. It is well recognized that adenosine is involved in lung inflammation. It exhibits cellular pro- and anti-inflammatory characteristic that contribute to the pathogenesis of respiratory conditions such as COPD and asthma [38]. Adenosine deaminase (ADA) is critical enzyme that plays a major role in the metabolism, which controls the amounts of adenosine [39]. There have been reports of serum ADA activity change in number of lung disease, including COPD and tuberculosis (TB) [40]. T-lymphocyte growth and proliferation in the thymus are induced by ADA compared to erythrocytes; lymphocytes contain ten times more of it [41].
Ramasamy R *et al.,* [42] demonstrated an elevation in smoker's adenosine deaminase activity compared to healthy non-smokers. This observation suggests a potential involvement of ADA in mediating certain chronic health risks associated with smoking like TB and COPD, and which are linked to increase in ADA levels. The study also reported a positive correlation between activity of ADA and pack size. While Bhagwan *et al.,* [43] discovered a notable reduction in ADA activity in serum of both COPD patients and healthy smokers compared to healthy non-smokers. Yanyan *et al.,* [44] noted a significant influence of smoking on serum ADA activity in individuals with acute coronary inflammatory (ACI), they observed that individuals with ACI, who also are alcoholic and smokers had reduced serum ADA activity; in contrast, diabetes and hypertension had the reverse impact. Additionally, chao Xuan *et al.,* [45] illustrated a significantly reduction in activity of serum ADA among individuals with coronary artery disease (CAD), particularly in those with myocardial infraction (MI). Certain authors [46, 47] demonstrated a significant reduction in serum ADA levels among individual diagnosed with head and neck cancer, which raises the possibility that activity of serum ADA perhaps helpful for both the disease's diagnosis and follow-up.

When tongue squamous cell carcinoma (SCC) advanced from stage one to stage three, there was a noticeable elevation in serum ADA activity [29], ADA increase considerably, so saliva ADA may help in the early detection of tongue SCC [48]. On the other hand, no such notable variations were seen when Saracoglu *et al.,* [31] examined the salivary activity of 5`-nucleotidase (5`-NT) and ADA in cases of oral leukoplakia. Pia Lopez *et al.,* [49] observed

no differences between the oral potentially malignant disorders group and control, neither did they find any significant differences in salivary ADA.

During periods of intense skeletal muscle activity, when ATP consumption outpaces its regeneration, the adenine nucleotide metabolism shifts towards the accumulation of ADP and AMP. The energy produced by ATP hydrolysis is reduced when the ATP/ADP ratio is lowered. the ability to effectively contract muscles is extended by AMPDA's conversion of adenosine monophosphate to ammonia and IMP, which when paired with reaction of adenylate kinase, restores high ATP/ADP ratio [50]. In cardiac muscle, the metabolic role and effects of AMPDA activity are distinct. The production of guanine nucleotides and regulation of the adenine nucleotide pool are the two functions of AMPDA activity in the well-oxygenated heart muscle.

Interestingly, atrophic skeletal muscle is associated with lower levels of adenine nucleotides, particularly ATP, and increase expression of AMPDA isoform 3 (AMPDA3) which is seen with cancer cachexia, diabetes, and chronic renal disease [51]. According to Davis *et al.,* [52] AMPDA3 may have an effect on reducing the rate of protein breakdown in certain long-term illnesses. Since increased AMPDA3 level do not lower rate of protein synthesis, increasing AMPDA activity further may be a desirable therapeutic target for conditions where muscle protein breakdown and subsequent muscular atrophy are the hallmarks of disease. Faridah *et al.,* [53] observed that serum ADA, AMPDA were significantly decreased in patients with renal stones when compare to control group.
To the best of our knowledge, previous studies have not established correlations between smoking and AMPDA activity. Therefore, we hypothesize that enhancing the efficiency of enzyme may be attributed to the impact of cigarette smoking on lungs and induce hypoxia so it effects the cardiac ischemic that's lead to adenylosuccinate synthetase's low cardiac activity prevent the IMP that is generated from being reincorporated into adenine nucleotides under ischemic conditions, even though the flux through AMPDA increases as AMP concentration rises. The majority of IMP that is produced in thus discharged from the cell after being further broken down to inosine. This indicates that AMPDA and 5`-NT competes with each other for AMP, which will decrease the production of protective adenosine [54]. Many molecular and signaling pathways are regulated by phosphate, AMPDA activation is also linked to intracellular phosphate depletion. The AMP-dependent enzyme AMPDA lowers nucleotide pools by converting AMP to IMP [55].
Recent studies have shown that cigarette smoking effects significantly on hematological parameters, smokers exhibited higher levels of WBC, RBC, Hb and MCV compered to non-smokers [56, 57]. The result in present study demonstrates a notable decrease in platelet, while some research found no significant difference in platelet counts between smokers and non-smokers [58, 59]. However, other research, including Wazzan *et al.*, (2020), observed a significant increase in the mean platelet count among smokers [60]. Our result shows an elevation in MCV level, and this result agree with previous results [61, 62]. Zahraa *et al.,* [63] suggested that increase in MCV and other hematological parameter may be linked to increased risk of COPD, atherosclerosis and cardiovascular disease.

Conclusion

The current study indicates that serum and saliva ADA and AMPA levels increase in smokers due to effect of nicotine, the primary component of cigarette smoke. These finding are strongly suggestive of role of saliva AMPDA in initiated diagnostic of tongue cancer and role of ADA in diagnosis lung disease and coronary artery disease due to prolonged smoking.

Further research is needed to elucidate the exact physiological and pathological roles of salivary ADA and AMPDA in smokers, as the current understanding remains incomplete.

5. Disclosure and conflict of interest

The authors declare that they have no conflicts of interest.

6. Ethical approval

All experiments were followed in accordance with Helsinki Declaration of 1975, as revised in 2000. This work has been approved by the ethical committee at the University of Technology and gives their informed consent to the work.

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