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Oral Manifestations, Biochemical, and IL-6 Analysis of Saliva in Epileptic Patients under Treatment

Dr. Fawaz Dawood Al-Aswad, B.D.S.,M.Sc.,Ph.D. *
Dr. Omar Faridh Fawzi, B.D.S. M.Sc. **

Abstract

Background: From the ancient Greek (epilepsia). Seizure is common and diverse set of chronic neurological disorders characterized by seizures.

Patients and Methods: The sample population comprised 10 epilepsy patients and 10 persons with no history of systemic disease, oral manifestation was recorded as well as saliva samples were taken. The IL-6, alkaline phosphatase and total salivary proteins had been measured.

Results: The most frequent oral manifestation was gingival hyperplasia 85.7%, and dry mouth 75%, increased level of IL-6 and total salivary proteins in epilepsy patients receiving treatment, when comparing the level of alkaline phosphatase, slightly decreased in study group, with no statistical significance.

Conclusion: In addition to the systemic effects of antiepileptic medication, and oral effects, including saliva composition and oral manifestation was recorded also. Therefore epileptic patients are at an increased risk of developing dry mouth, gingival hyperplasia, and alteration in saliva composition compared with health subjects.

Keywords: Epilepsy, Oral Manifestations, ALP, TSP, IL-6.

Introduction

The word epilepsy is derived from the Greek word "epilambamein" meaning to take or to seize. Between 400BC and 200AD Hippocrates, Aretaeus, Celsus, and Plinius all provided careful description of major and minor seizure. Hippocrates even recognized that seizures originated in the brain [(1)].

Many diseases that involve the whole body produce oral changes at some stages in their course, the oral manifestations are often mile and over shadowed by the major manifestation of systemic disease [(2)].

The most commonly used laboratory diagnostic procedures involve the analysis of the cellular and chemical constituents of blood, other biological fluids can also be utilized for the diagnosis of the disease and saliva offers some distinctive advantage over serum because it can be collected non-invasively by individuals [(3)], also saliva may serve as a feasible fluid to plasma in order to be used as surrogate for the free concentration of different enzymes and interleukins.

Early reports in the literature urge dentists to be aware of the possible oral manifestation of the epilepsy also several salivary constituent were

* Assistant Prof. Department of oral medicine, college of dentistry, University of Baghdad.
**Assistant Lecturer, Department of oral medicine college of dentistry, Al Yarmouk University.

assayed in saliva from epileptic patients who were using different anti-epileptic drugs. Phenytoin, valproate, and carbamazepine, and were compared with levels in un-mediated health control subjects.

In spite of the this fact, no study has been conducted to reveal the effects of anti-epileptic medications in Iraq, hence this study aimes to determine the oral health states of epilepsy patients and to gain further insights into composition of saliva in epilepsy patients.

Materials and Methods

Ten patients taking Tegretol in regular doses for less than 6 months participated in the study, ten healthy looking, according to their personal statement. The epileptic patients were diagnosed according to the criteria used in the department of psychiatry in Al-Yarmouk teaching hospital by neurologist specialist; depending on the health history and examination findings, laboratory work may be ordered, this might include blood studies and special testing such as EEG, CT, MRI, because EEG procedures usually performed between seizures, they were examined from the period (1/10/2012---9/11/2012) to detect the prevalence of oral manifestation, salivary biochemical analysis, and IL-6 measurement.

Exclusion criteria

-Newly diagnosed patients not receiving treatment.

-Patients with severe periodontal disease.

-Any other systemic disease.

All the patients examined by a single examiner under standardized conditions, the procedure of examination was done in sequence according to the directions suggested by the W.H.O.(1987).

To avoid circadian variations, saliva sample was collected between 9 AM and 10 AM. In order to obtain sample of total saliva, the patients were instructed not to eat or drink except water for one hour. The method of wu-wang[4] was used for saliva collection.

Seven to eight ml of un-stimulated saliva collected after asking the individual to rinse his or her mouth with water the saliva collected into small plastic polyethylene cups, stored for about 2 weeks before analysis at (-15 to -20), the collected saliva centrifuged at 1500 RPM for 10 minutes, the supernatant later used for biochemical studies including:

1-Alkaline Phosphates ELISA kit catalog no.(SB-E09033h).

2-Total salivary proteins cat#23225.

3.Interleukin-6 ELISA kit catalog no.ABIN455601

Total salivary proteins level was determined using BCA technique.

Statistical Analysis

Statistical analysis was performed using descriptive data analysis.

1- Tables (Frequencies, Percentages and Cumulative Percentages)
2- Mean, Trimmed Mean, Median.
3- Standard Deviation (Std. D.), Standard Error (Std. Error), Range, Interquartile range.
4- (95%) Confidence interval for population Mean values.
5- Two Extreme values (min. and max.) respondents.
6- Contingency Coefficients for the causes correlation ship of the contingency tables.
7- Graphical presentation by using:Bar- charts, Custer Bar Charts.Pie - charts.Stem-Leaf Plot.
8- Inferential data analysis.

All data on disease stated were analyzed by using:

1. Binominal test procedure.

2. Mann-Whitney test (a non-parametric equivalent) to the test.
3. Contingency Coefficients test.

Results

Table (1) shows the association causes correlation ship between the two samples of (epileptic statues), (study and control) according to their demographical characteristics variables (age per year), (gender, age of onset per years) through their different levels, the results had been indicated that there were a non-significant differences at $p>0.05$ for the observed frequencies distribution of the two samples which were corresponding proportionally. These would be recommended and extremely reliable for the studied samples. In other words we can conclude that there characteristics variables between the two groups and that established/or adapted the two independent samples for studying the phenomena.

Distribution of the studied samples according to age, gender, treatment, age of onset, and duration of treatment.

A. The sample consist of 10 patients receiving treatment for epilepsy, the age range of these patients was (20-60 years), there was 7 (70%) males with the mean age of (33.8), and 3 (3%) females with the mean age of (30.6), and table (2), figure (1) show the distribution of the studied sample according to age and gender.

B. The second group is control group similar in respect to age, gender, and ethnic matched with epilepsy group, there were 10 healthy looking individuals, and have no history or clinical evidence of any disease or obvious abnormalities.

C. 4 (40%) patients treated with Tegretol and 6 (60%) patients treated with sodium valproate, again table (2) demonstrates the distribution of patients according to the duration of treatment and the age of onset.

To predict or studying the causes correlation-ships among some related variables distribution to the studied oral manifestation, the results have reported that a non-significant difference was obtained with the distribution of the two categories (yes or no) for oral manifestation (gingival, hyperplasia, dry mouth) for both the study and control group. (Table (3)

Table (4) represents the target of the critical base line of the study sample and control, the results of multiple comparisons by Chi-Square. method which represented statistically non-significant differences at all parameters (ALP, IL-6, and TSP)

In figure (3,4) there were differences in the salivary IL-6 and TSP between study and control groups, which illustrated the greatest value with study group, while figure (2) showed the lowest value in studying group when comparing with control.

Table (5) showed distribution of the sample according to the type of medication (Tegretol, sodium valproate) with a

comparison`s significant to different parameter, a non-statistical significant result for ALP, IL-6, and TSP showed between the two groups.

In figure (5,6) there were differences in the salivary ALP and IL-6 between the two independent epilepsy status treatment, the greatest value with the Tegretol group, while figure (7) illustrated the highest value of TSP in epilepsy patients treatewith sodium valproate.

Discussion

In this study attempts have been made to assess oral health status of epileptic patients, the study had two limitations, first we could not use x-ray imaging, and the second was the lack

of literature on the subject at a country level for comparisons and discussion purposes. Anti-convulsant drugs can cause pathological changes in the mouth, the patient may have following signs and symptoms dry mouth, irritation, or soreness of the tongue and mouth, red irritated or bleeding gums, and swelling of the face, lips, or tongue.[5,6,7,8]

Other possible side effects of medications may include bone loss which can lead to osteoporosis over the long term of use.[9]

The most common oral finding found in this study was gingival hyperplasia and dry mouth, this disagreed with that evidenced by **Gary et al., (2011)**[10] , they stated that burning mouth syndrome present the challenges complained for epileptic patients.

Different between this type of study and other studies is not surprising taking into consideration the methodological quality of included studies was assessed on the basis of method of allocation concealment, loss of participant size sample and outcome concealment.

So far, it has not yet been investigated whether epilepsy may play a role in oral manifestation pathogenesis, however there is limited to moderate evidence supporting the efficacy of commonly used anti-convulsant for treatment of patient with some of the manifestation like burning mouth syndrome.[11]

No oral mucosal lesion was detected, while carbamazepine side effect have been linked to licheniod reaction in the oral mucosa by other study[12], other reported that gingival hyperplasia was the most common oral manifestation.[13,14]

In recent years increasing evidence has indicated that immune and inflammatory reactions reflected in saliva in various diseases. Furthermore, inflammatory processes such as the production of pro-inflammatory cytokines and related molecules, have described in saliva after seizure, although clear evidence indicates the pro-inflammatory cytokines can affect the occurrence of seizures[15,16], similar to that repointed by **Limataien et al., (2009)**[17]. The increase in the level of the interleukin 6 may be explained by the fact that activation of the immune system and associated inflammatory reactions in saliva may mediate some of the molecular structure changes occurring during and after seizure activity.

This observation shows that immune-like mechanisms can be triggered in CNS by seizures, thus challenging neuroscience research to investigate in more detail the mechanisms underlying this activation and its functional consequences. Whether immune responses that take place in epileptic saliva is beneficial or noxious to the patient is still an open and intriguing question that should be addressed by further investigation.

Key open questions include whether those pro-inflammatory signals represent a more epiphenomena or if they are significantly involved in the etio-pathogenesis of seizures, and possibly contribute to epilepto-genesis.

Human saliva contains proteins that can be informative for disease detection and surveillance of oral health [18]. This data suggest that use of anti-epileptic drugs may result in increased levels of salivary proteins, which involved in the proteins of the oral cavity against microbial infections.

There was no significant difference when the mean of the patients' saliva ALP compared with that of the control. Again, up to our knowledge, this study was the first to measure ALP activity in saliva of epilepsy. While there was no significant difference when the

mean of the patients` serum ALP was compared with that of the control.[19,20]

On the other hand there was significant correlation between serum total ALP activity in patients receiving carbamazepine therapy as compared to control in other studies.[21,22,23,24]

The results showed that our production of ALP and IL-6 in epilepsy patient receiving carbamazepine as compared with those with sodium valproate treatment, the result may reflect a chronic immunological and biochemical process more preventive in patients with carbamazepine as compared with those receiving sodium valproate.

Conclusions

The most frequent oral manifestation in epilepsy patients was gingival hyperplasia the result reflect immunological reactions as salivary IL-6, and TSP increased in epilepsy patients compared with healthy control, while ALP was shown to be higher in control patients compared to epilepsy patients receiving treatment, these evidences opened the doors to additional questions of whether these biochemical and immunological changes may enhance the brain tissue to develop seizures

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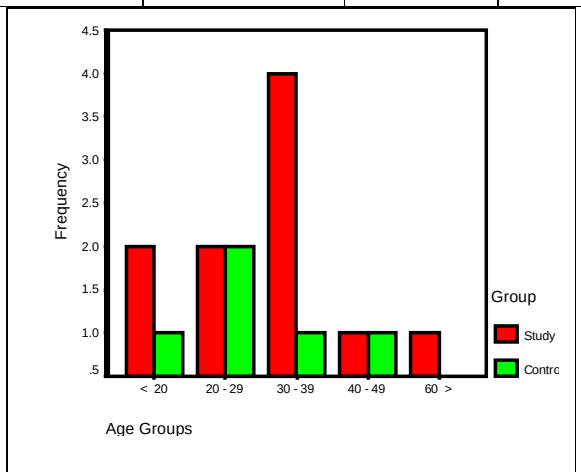
Table (4-1): Distribution of some demographical characteristics variable's tests between the two independent samples of Epileptic status

Pervious History of Patients	Contingency Coefficient	P-value	C.S.
Age groups	0.315	0.800	NS
Gender	0.333	0.171	NS
Age onset	0.509	0.263	NS

Table (2) Distribution of the studied demographical characteristics of the study group of Epileptic status and some related variables with comparison significant

Variables	Groups	Frequency	Percent	Cum. Percent	C.S. P-value
Age Groups	≤ 20	2	20	20	χ^2= 3.000 P = 0.558 NS
	20 - 29	2	20	40	
	30 - 39	4	40	80	
	40 - 49	1	10	90	
	50 - 59	0	0	90	
	60 ≥	1	10	100	
Mean ± SD		31.00± 14.00			
Gender	Male	7	70	70	Binomial P=0.344 NS
	Female	3	30	100	
Treatment	Tegretol	4	40	40	Binomial P=0.754 NS
	Sodium Val orate	6	60	100	
Age of onset groups	≤ 20	3	30	30	χ^2=2.000 P = 0.736 NS
	20 - 29	1	10	40	
	30 - 39	3	30	70	
	40 - 49	2	20	90	
	50 ≥	1	10	100	
Mean ± SD		31.00± 14.19			
Duration of Treatment (per month)	1 (m.)	0	0	0	χ^2= 1.200 P = 0.753 NS
	2 _ 5 (m.)	2	20	20	
	6 _ 11 (m.)	2	20	40	
	12 _ 23 (m.)	2	20	60	
	24 _ 59 (m.)	4	40	100	
	60 > (m.)	0	0	100	
Mean ± SD		19.00± 16.00			
Gingival hyperplasia	No	4	40	40	Binomial P=0.754 NS
	Yes	6	60	100	
Dry mouth	No	4	40	40	Binomial P=0.754 NS
	Yes	6	60	100	



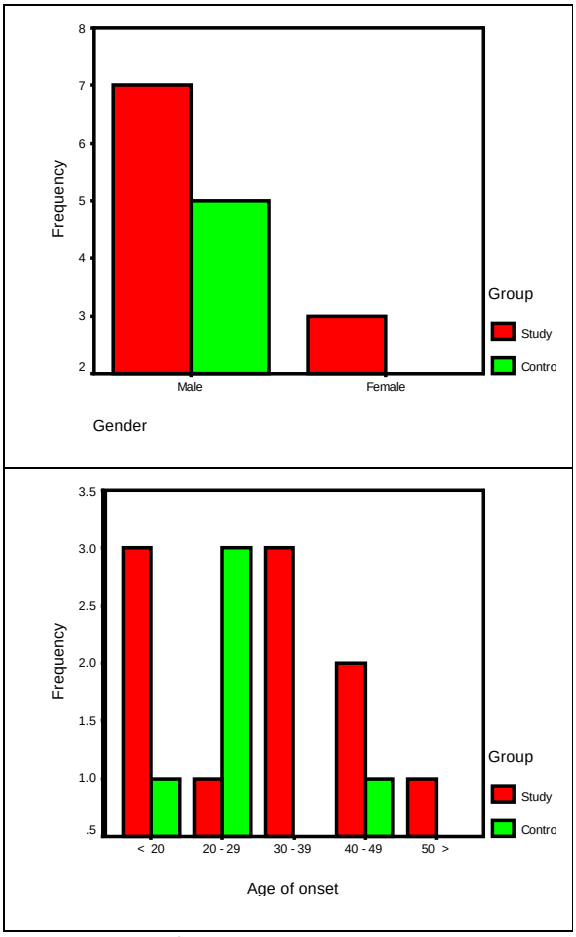


Figure (1): Cluster Bar charts of the two independent sample's groups of Epileptic status according to some related variable's distribution

Table (3): Testing the random distribution of the two independent epileptic status samples according to some related variables

Parameters	Group	No.	Mean	Std. Dev.	Std. Error Mean	C.S.(*) P-value
ALP ng/ml	Study	10	19.0	8.5	2.7	P=0.099
	Control	5	24.2	8.2	3.7	NS
IL-6 pg/ml	Study	10	33.8	37.9	12.0	P=0.513
	Control	5	22.4	4.1	1.8	NS
Total Proteins ng/ml	Study	10	1959.2	1217.7	385.1	P=0.055
	Control	5	975.5	584.7	261.5	NS

Table (4): Descriptive statistics of the two independent epileptic status sample's parameters with comparison's significant

Variables	Groups	Frequency & Percents	Group		Total	C.S. P-value
			Study	Control		
Gingival hyperplasia	No	Frequency	4	4	8	CC=0.354 P=0.143 NS Odds Ratio (1 : 6)
		% Gingival hyperplasia	50%	50%	100%	
		% Group	40.0%	80%	53.3%	
		% of Total	26.7%	26.7%	53.3%	
	Yes	Frequency	6	1	7	
		% Gingival hyperplasia	85.7%	14.3%	100%	
		% Group	60%	20%	46.7%	
		% of Total	40.0%	6.7%	46.7%	
Dry mouth	No	Frequency	4	3	7	CC=0.186 P=0.464 NS Odds Ratio (1 : 2.25)
		% Dry mouth	57%	43%	100%	
		% Group	40.0%	60%	46.7%	
		% of Total	26.7%	20.0%	46.7%	
	Yes	Frequency	6	2	8	
		% Dry mouth	75.0%	25.0%	100%	
		% Group	60%	40%	53.3%	
		% of Total	40.0%	13.3%	53.3%	

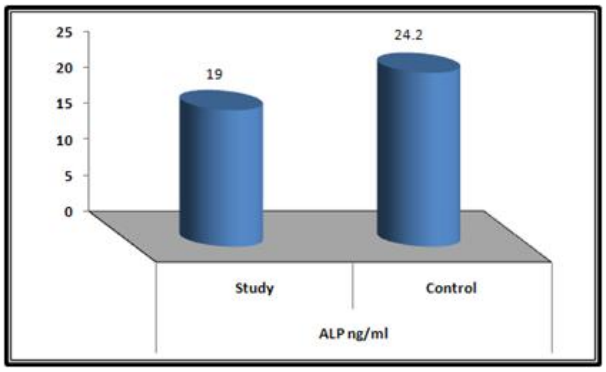


Figure (2): Bar chart for mean values of ALP (ng/ml) parameter for the two independent epileptic status samples

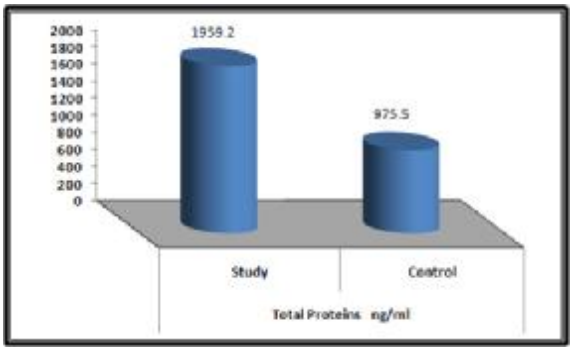


Figure (3): Bar chart for mean values of IL-6 (PG/ml) parameter of the two independent epileptic status samples

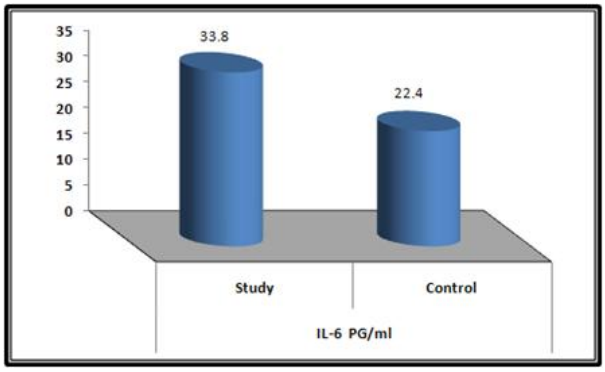


Figure (4): Bar chart for mean values of Total Proteins (ng/ml) parameter of the two independent epileptic status samples

Table (5): Descriptive statistics of the two independent epileptic status treatment's parameters with comparison's significant

Parameters	Treatment	No.	Mean	Std. Dev.	Std. Error Mean	C.S.(*) P-value
ALP ng/ml	Tegretol	4	20.3	12.0	6.0	P=0.915 NS
	Sodium Valporate	6	18.2	6.3	2.6	
IL-6 pg/ml	Tegretol	4	50.0	56.2	28.1	P=0.201 NS
	Sodium Valporate	6	23.0	18.6	7.6	
Total Proteins ng/ml	Tegretol	4	1455.8	643.0	321.5	P=0.240 NS
	Sodium Valporate	6	2294.8	1443.2	589.2	

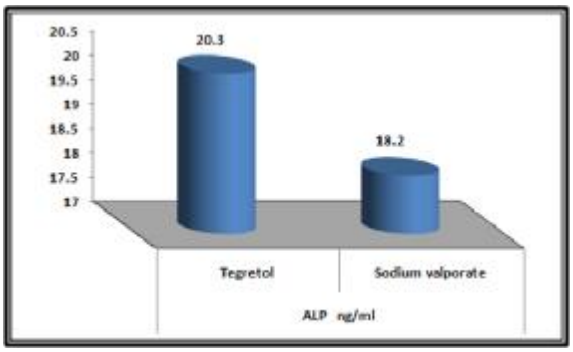


Figure (5): Bar chart for mean values of ALP (ng/ml) parameter for the two independent treatments of epileptic status

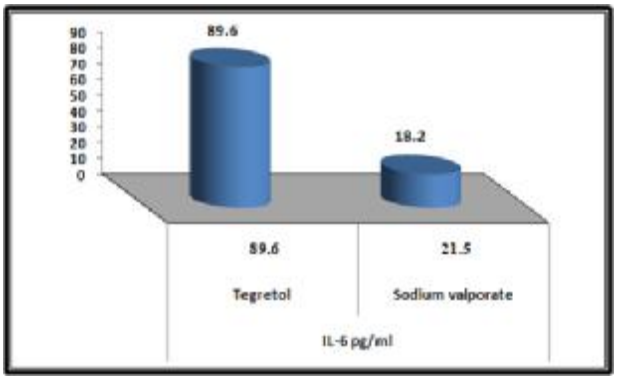


Figure (6): Bar chart for mean values of IL-6 (pg/ml) parameter for the two independent treatments of epileptic status

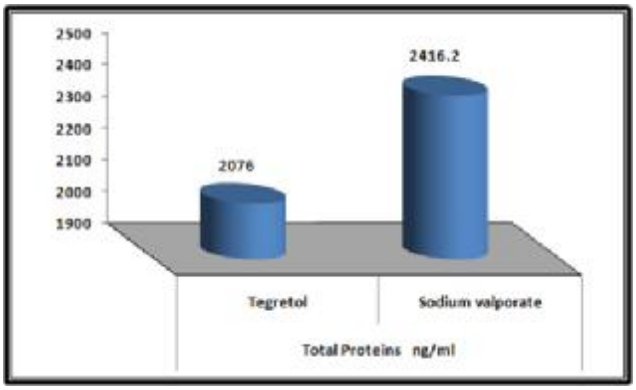


Figure (7): Bar chart for mean values of Total Proteins (ng/ml) parameter for the two independent treatments of epileptic status