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THE IMPACT ROLE OF USING DENTAL AMALGAM "SILVER" FILLINGS ON THE CHOLINERGIC AND ENDOGENOUS ADRENERGIC ACTIVITY

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ABSTRACT

The effect of silver dental amalgam fillings on cholinergic activity was investigated through measurement of AChE activity using UV-Vis spectroscopy by Ellman modified method. Results demonstrated that the enzyme activity decreased significantly in students have 1-2 fillings more than that observed in subjects have 3-5 amalgam fillings which still under control values. For long time exposure to the fillings (4 years), the enzyme activity diminished annually among young undergraduate male students (18-25 years old) when compared with control subjects. In addition, salivary α-amylase activities in the first group (1-2 mercury fillings) were none significantly lower than control subjects. Furthermore, serum Hg concentrations in target subjects were considerably higher than those without fillings. In conclusion, according to the changes observed in AChE activity by time of exposure and higher concentration of Hg, it is proposed that: increasing duration and number of amalgam fillings might in fact be capable of decreasing cholinergic activity whereas only slightly changes in adrenergic activity.

KEYWORDS: **Amalgam filling, AChE activity, Amylase activity, Heavy metal ions level.**

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INTRODUCTION

In most parts of the world, dentists are still using silver dental amalgam fillings in our teeth. This filling consists of a mixture of Hg 50%, Ag 35%, Sn and other metals 15%[1]. Clinical experience had identified hundreds of thousands of those with several amalgam filling have Hg levels about 10 times than those without, and their saliva and excretion levels decline about 90% after amalgam replacement, and this will approve the growing evidence of putting so much Hg filling is not safe[2]. It is worthy to mention to the clear conclusion of the World Health Organization (WHO) in 1990, which serves that Hg in amalgam filling is the primary source of human exposure to toxin[3]. Several studies of the National Institute for Health detected a significant correlation between the number of dental silver fillings and epilepsy, migraines, mental disorders and the nervous system diseases[4]. Other studies have found that Hg is a neurotoxin agent and is able to kills or damages brain cells and nervous system[5-8], where it inhibits the calcium-dependent neuro-transmitter release leading to inhibition of neurotransmitters production[9, 10]. A large epidemiological study documented that Hg causing depression and mood disorder by its mechanism on lowering the levels of neurotransmitters dopamine, serotonin, norepinephrine and acetylcholinesterase enzyme (AChE)[11, 12]. The enzyme AChE (EC. 3.1.1.7) is a serine protease, found mainly in neuromuscular junctions and cholinergic brain synapses. It conducts tissue such as: nerve and muscle, central and peripheral tissues, motor and sensory fibers[13, 14] where its activity serves to hydrolyze the neurotransmitter acetylcholine to acetate and choline at the cholinergic synapses (cholinergic activity). Terminating nerve impulse transmission, though, it's a regulating of cholinergic neurotransmission in mammals, birds, fish, and insects[15]. Previous studies had documented the use of AChE activity as biomarkers of pollution[13,14,16-18]. Interestingly, amounts of organic and inorganic Hg in the whole saliva had been significantly indicated to be higher in subjects with dental amalgam fillings compared to the non-amalgam groups. Furthermore, there is a correlation between the organic and inorganic Hg with the number of filled tooth surfaces[19]. Chatterton`s investigation, RT in 1996 was successful to record that the concentration of salivary α-amylase might be used as a measure of endogenous adrenergic activity of human beings under stress [20] and as a good indicator of mental stress[21]. Alpha-amylase (α-1,4 glucan-4-glucanohydrolase, EC 3.2.1.1) is widely obtained in mammalian tissues, microorganisms and plants. Its main activity serves the hydrolysis reaction of α-D-(1,4) glycosidic-linkages of starch components (amylose and amylopectin), glycogen and various oligosaccharides. In mammals and moreover, there is an increasing number of previous investigations had evaluated the possible inhibition efficiency of α-amylase to treat diabetes[22]. However, because Hg is easily absorbed (80%) when it inhaled, part of it might dissolved in saliva, and a little quantity of which is absorbed in the gastrointestinal tract. Many amalgam fillings in our teeth, establish the major cause of display to inorganic Hg[23,24]. The objective of this work was to determine how the uptake of silver amalgam filling had a possible hazard effect on the CNS and adrenergic activity. That was by detecting the alteration of AChE and salivary α-amylase activity, and measuring the concentration of Hg in serum of Chemistry students who have numbers of amalgam fillings. Consequently, data were compared with other without fillings as control, and then statistically analysed.

MATERIALS AND METHODS

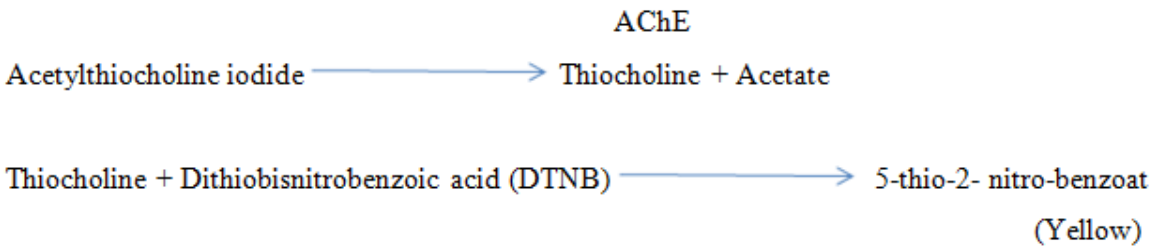
(i)Study population

Serum AChE was supplied by 78 undergraduate students of Chemistry Dep. Al-Mustansyriah University, where the study performed up to 1 year (2012). Twenty nine of them were exposed to 1-2 amalgam fillings in their teeth (1st group), and the 2nd group was having 22 students with 3-5 amalgam–filled tooth surfaces. The rest were without any type of fillings (control). All groups were out of any pathological, neurological or cholinergic disorder by the report of our

University Medical Center physicians, neither smoking nor alcohol consuming, and having their fillings since 4 years and below. Ethical consent was considered with all cases. Mean age was 23 years old (ranged from 18 to 26 years old). Blood was sampled between (8:30-10:30 am) in a collection tubes, immediately centrifuged for 10 min at 1500x g (3000 rpm), sera were collected in tubes without anticoagulant and stored in -10^{0}C until analysis for AChE activity on the next day. All chemicals used obtained from Sigma and Merck chemical companies (Germany).

(ii) AChE assay

AChE activity was determined at 25°C by the spectrophotometeric method of Ellman *et al*[25], based on the following colorimetric reactions:



(iii) Salivary α-Amylase collection and assay

Preparation and handling saliva of the 1st group was done according to a standard procedure[26]. The preferred saliva was collected in a non-citric acid plane tubes after gum chewing for 10-15 min., then Saliva samples frozen up to -4^{0}C prior to assay to precipitate the mucins. On the day of assay, thaw completely, well vortexes, and centrifuge at 1500 x g (3000 rpm) for 15 minutes. The reaction carried out on a clear sample after centrifugation.

(iv) Serum Hg concentration

Sera used for AChE analysis were served to determine Hg concentration as well. Preparation of reagents and samples of all groups under study was done according to a standard procedure[27]. Measurement of Hg^{+2}concentrations (µg/L) was carried out with a newly developed cold vapor flow-injection mercury system (FIMS 100) with cold-vapor atomic absorption from the Shimadzue 6800 at the Ministry of Science and Technology/ epidemiological research office-Baghdad.

(v) Statistical analysis

Results were expressed as mean ±SD. The significance of differences was assessed Students *t*-test for groups of un-paired observations. Differences were considered significant if $P<0.05$, and frequency and ratio occurrences of each group were calculated. All the statistical analysis was performed by SPSS, Version 16software.

RESULTS

Serum AChE activities

The major characteristics of amalgam dental fillings and control students in our study were compared in Table 1, The prevalence rates of 1-2 fillings (56.8%), and whom had 3-5 fillings in their teeth (43.1%) were occured around 18-26 years old (23.18 ± 1.75 and 23.00 ± 2.19, respectively). Also, 58.6% and 41.3% of the 1stgroup had their fillings up to two and four years respectively, similarly, higher proportion of the 2nd group suffered fillings up to two years (54.5%) rather than those of four years filligs (45.4%). Results had clearly indicated an alteration of AChE activity obtained by dental fillings people, which went on decreasing as duration of fillings increased. Principly, 60.7% of AChE activity decreased significantly from the initial activity of non-fillings students (39.2%), (3.68±1.03 *vs.* 4.77±0.92 U/L.min, $p<0.001$), Table 2. Students of the 1st group showed an initial decrease in AChE activity than control, while 2nd group exposed students showed about a 1/3 fold increase in enzyme activity than the 1st group, however, still it

remained significantly lower than control (3.38±1.06 < 4.107±0.85 < 4.77±0.927 U/L.min, respectively, p<0.02), Fig 1. At all tested filling groups, the enzyme activities were observely annually diminished ($1^1/_4$ fold) until reached to the lowest activity after four years exposure, compared to controls. For students having fillings for at least one year, the highest activity of the enzyme was 4.33±0.41U/L.min. For those with fillings up to two years, the activity was lower (3.60±0.42), and gradual low activity observed up to three years exposure pule (3.44±0.11). After four years exposure, the activity was further reduced, and shown to be 3.36±0.14 U/L.min, which is even below than control (4.77±0.42), Fig 2.

2. Salivary α-amylase activity

The enzyme activity in the tested 1st group was not significantly lower than that in subjects free of amalgam fillings (p>0.05), Table 2, and Fig 3.

3. Serum Hg^{+2} concentrations

Serum Hg concentration was significantly higher in the 2nd group than that in the 1st and control groups (Table 3,Fig 4).

Table 1.Characteristic of students included in this study.

All groups seem to have the same age ~ 23 years. As a mean, 17 students from the first group (1-2 fillings) had their fillings within 1 year and 39 days and 12 students were having their fillings within 3 years and 31 days. Twelve students of the 2nd group were having their fillings within 1 year and 25 days, and 10 students were exposure to filling within 2 dears and 84 days.

Characteristic	(1-2) Fillings group			(3-5)Fillings group			Without fillings group		
	Mean±*SD*	No.	%	Mean±*SD*	No.	%	Mean±*SD*	No.	%
Age (years)									
18-26	23.18±1.75	29	56.8	23.00±2.19	22	43.1	22.63±2.3 2	33	100
Fillings time (years)									
1-2	1.39±0.49	17	58.6	1.25±0.58	12	54.5	-	-	-
3-4	3.31±0.45	12	41.3	3.25±0.42	10	45.4	-	-	-
Total	2.40±1.09	29	99.9	2.84±1.06	22	99.9	-	-	-
No. of amalgam fillings	1.26±0.49	29		3.8±0.80	22		-	-	-

Table 2.Enzymesactivities in different groups of students.

AChE activity was significantly decreased in amalgam filling group when compared to controls, while α-Amylase was non-significantly inhibited.

Enzyme tests	Amalgam fillings groups			Controls		
	Mean±*SD*	No	%	Mean±*SD*	No	%
AChE (U/L.min)	3.68±1.03**	51	60.7	4.77±0.92	33	39.2
α-Amylase (U/L.min)	37.87±15.43	29	60.7	38.12±11.28 *(N.S)*	33	39.2

*** P<0.001, N.S non significante*

Table 3. Serum Hg^{+2} concentration in µg/l at all groups of students.

Mercury level was significantly elevated in the 2nd group (3-5 fillings) more than the 1st group (1-2 fillings) when compared with control.

	(1-2) Fillings group		*(3-5) Fillings group*			*Without fillings group*		
	Mean±SD	*No.*	*Mean±SD*	*No.*	*%*	*Mean±SD*	*No.*	*%*
Hg^{+2} Conc.	*11.18±1.49****	*29*	*15.63±1.69****	*22*		*2.71± 0.91*	*33*	

**** p<0.0001*

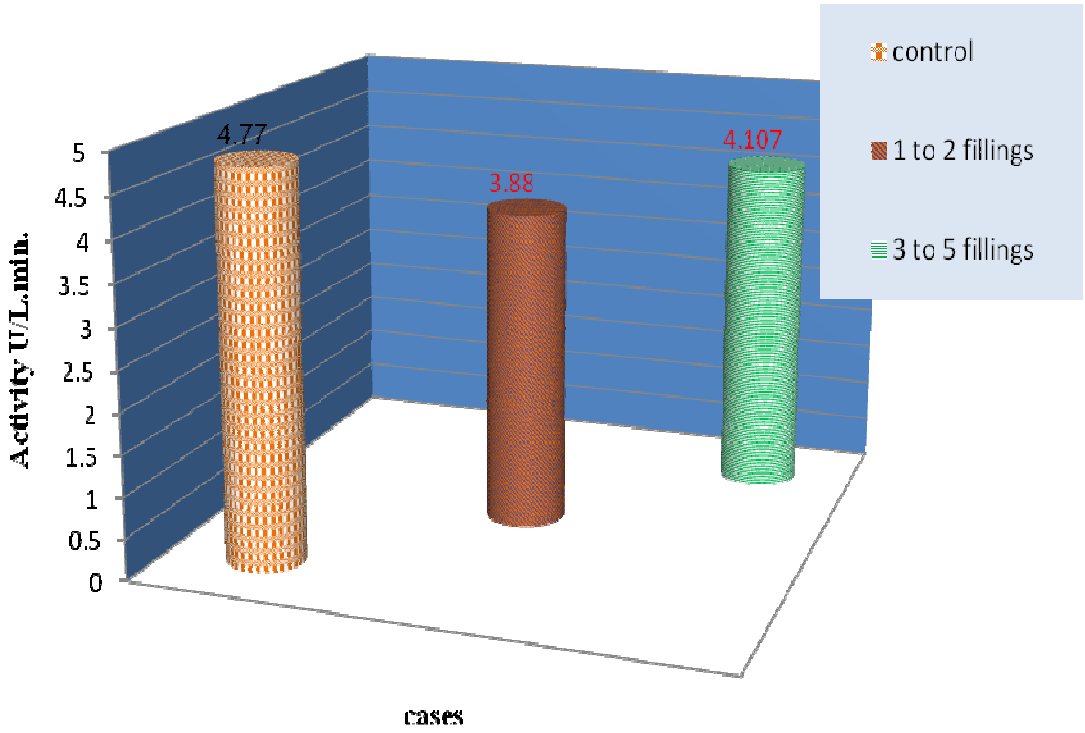


Figure 1. Serum AChE activity in amalgam filling subjects related to number of fillings.
The enzyme activity was lower in 1st group than that of 2nd group if compared to control.

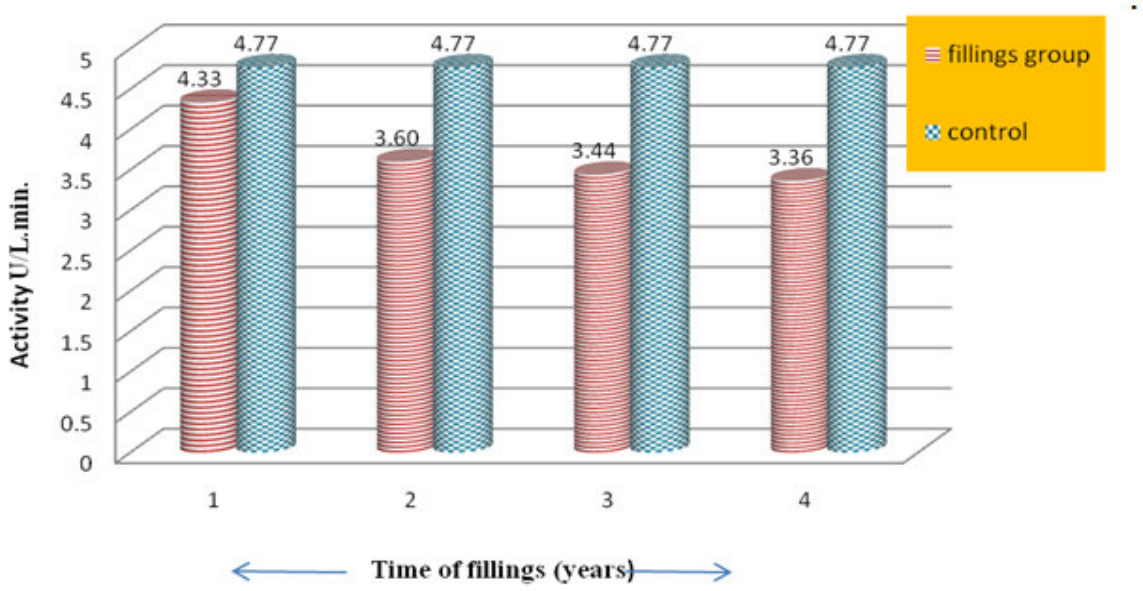


Figure 2. Serum AChE activity in mercury filling subjects related to time (years).
The enzyme activity was diminished by time until it reached to a low level after 4 years expusure when compared with controls.

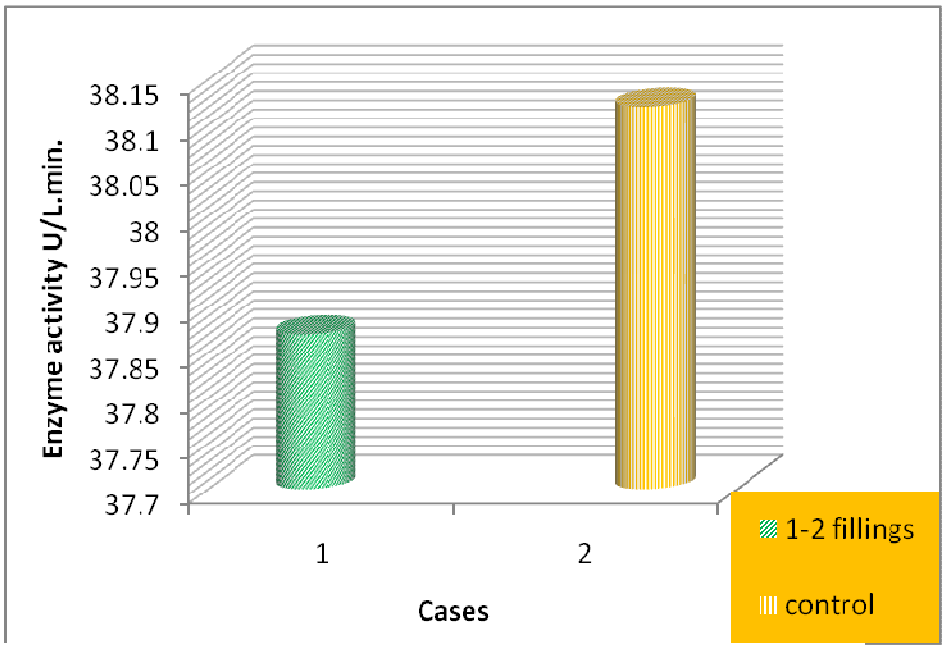


Figure 3. Alpha-amylase activity in subjects with 1-2 mercury fillings and without fillings.
The enzyme was non-significantly inactivated when compared with control.

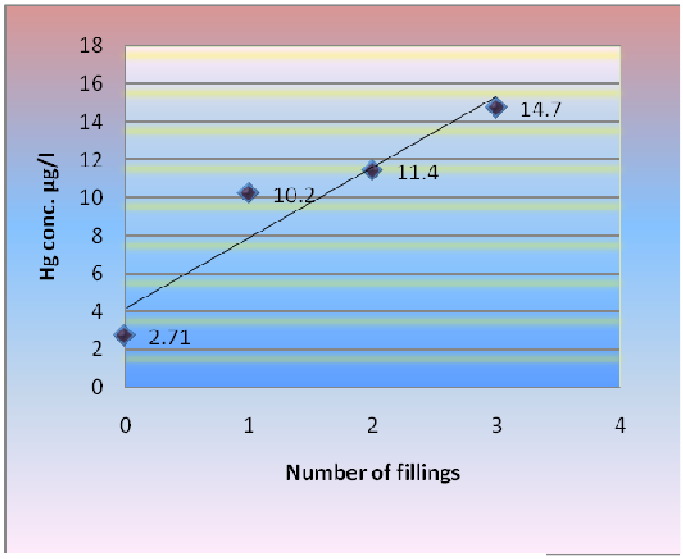


Figure 4. Serum Hg^{+2} Conc. in amalgam fillings and non-fillings subjects.
Leves of Hg were increased linearly as the number of fillings increased, when compared with control.

DISCUSSION

Our results strongly proves an inhibition effect on serum AChE activity as an immediate response to heavy metal pollution, which then decreases on extended exposure to Hg found in the dental amalgam–filled tooth surfaces. Our laboratory findings in this investigation are at variance with the dental profession opinion, which conforms that amalgam tooth fillings are safe on health. From letratures point of view, dental amalgams can be a major source of chronic Hg exposure and the use of amalgam fillings was totally considered unsafe[28]. In cholinesterase, Hg particles were found to be linked to histidine, carboxyl and methionine side chain, as evidenced by the complex structures with Hg[29], in addition, Hg reacts with S–S bonds found in in three intra chain in cholinesterase, leading to their disruption (R-S-Cl + Cl-Hg-S-R)[30, 31]. The R-S-Cl moiety may later be oxidized by another Hg^{+2} to form R-S-Hg-Cl. Thus the cleavage of S–S bridges by

$HgCl_2$ enables disturbtion the tertiary structure of proteins, lower their stability and hence to inhibit the enzyme activity. One can notice that a significant inhibition rate occur with 1st group population of our study, but surprising about the elevation of AChE activity observed in the 2nd group, Fig 1. The putative mechanism of this inhibition phenomena perhaps occur as a consequence of the cross linking of two coordinating residues present at the surface of the protein with Hg within the same enzyme molecule, thereby inducing a conformational change, which can lead to partial unfolding followed by irreversible denaturation, then inhibition occurs in the mille molar range and follows a slow binding mode. Mercury may also cross-link residues belonging to different enzyme molecules, leading to enzyme aggregation depends on protein and Hg concentration, it might therefore protect the enzyme from unfolding and do not induce conformational changes. In other words, it maybe argued that the intermolecular cross-links of Hg protect the enzyme from the intra molecular[30 -32]. In the present study, the noticeable decreasing in AChE activity seen after four years of having fillings, Fig 2, points to the fact that prolonged exposure inhibits the enzyme activity. Hence, this inhibitory effect indicated couldn't be ruled out, especialy when similar findings found by other researchers[32] had shown a decline in enzyme activity as the duration of exposure increased. This and previous results[28] confirm our current result where AChE was inhibited by Hg. The loss of AChE activity might be a toxic marker and may play a role hereditary predisposition of cholinergic disease. Salivary α-amylase, which consists of 496 amino-acid residues with one Ca^{++}, one Cl^- and 170 water molecules, considered a major constituent of human saliva. It plays a role in the early steps of starch digestion and may be involved in the colonization of bacteria participated in the formation of early dental plaque[33]. The results obtained here (Table 2, Fig 3), are consensus study by others[34,35], where α-amylase enzyme losses its activity in the presence of heavy metals Hg and Ag. The attribution of enzyme inactivation is either to the possibility of Hg ability to bind catalytic residues on the enzyme molecule surface, or to the replacement of Ca^{+2} from the substrate-binding site of the enzyme[36]. Herein, the general characteristic of the enzyme is related to its content of calcium ions (each enzyme molecule have one to ten Ca[37], and is known to have a role in binding of substrate. As a result, any molecule linked to the Ca binding site will becoming as a good as an enzyme inhibitor. Amylase always circulates in its active state around the body. In other words, it is not weakly inhibited as are the serine enzymes, although there are amylase inhibitors in wheat and anionic detergents can deactivate amylase, as do heavy metals[38]. Additional evidence, let us turn over another clear observation illustrated in Fig 4, where Hg concentration was elevated as the number of fillings was highly used by students. This result is supported by Nylander, *et al.*[39] where Hg accumulation in brain cells after exposure to amalgam fillings was evaluated, Fig 5. We know that inhibition of AChE causes neuropoisoning symptoms like headache, drooling, twitching, and severe inhibition might caused abdominal cramps, tremors, hypotension, asthma and brain hemorrhage. However, yet, a question mark will arise here when a professor of oral medicine, Spokesman, S.G., mentioned "*But that does not mean the population should not have amalgam fillings*"[40]. Identically, dentists are considering Hg amalgam is safe until otherwise proven, because it is unexpensive and had been in demand for over a century[41]. Afterwards, however, many studies introduced neurological, immune, and metabolic related disorders of Hg exposure and how it is able to damage brain cells and CNS[5,42-46]. Even more, British Dental Association (2013) recommended many tips to reduce using silver fillings in the foreseeable future and one should replace it as soon as possible. Likewise, It is worth mentioning the clear conclusion of World Health Organization[23], which found that Hg in amalgam fillings is the primary source of human exposure to toxin.

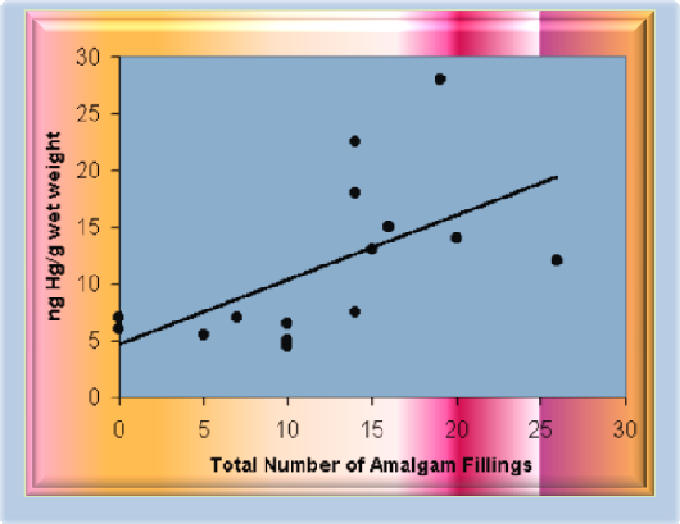


Figure 5. Hg concentration in human brain vs number of amalgam fillings.
The concentration of HG was increased in the brain tissues as the level of filling.

CONCLUSION

To sum up, our laboratory findings of inhibitory effect of Hg on serum AChE activity, which was increased linearly as a function of number of fillings and exposure time, seems clear cut and at variance with dental professions opinion, because loss of AChE activity may be a toxic marker which might play a role hereditary cause of cholinergic disease. Therefore, it is not only nonsense to consider safety of amalgam fillings, but criminally non liable and dentists should reduce using it as much as they can, according to our patients opinion.

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