IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 2, March 2022
Impact Factor: 6.252

# Chromogranin A in Human Saliva as Putative Biomarker of Alzheimer's Type Dementia

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**Abstract:** *Early diagnosis of Alzheimer's disease will be helpful as no clinical method is available to determine the role of mild cognitive impairment. Chromogranins are soluble glycophosphoproteins which activate microglial cells leading to neurotoxic phenotype. There is need for biomarkers through non-invasive approach to identify incipient Alzheimer's disease patients with mild cognitive impairment. Chromogranin A present in saliva samples was determined using ELISA. The immunoreactive patterns of Salivary CgA were assayed in dementia and compared to those observed in Alzheimer's disease. Salivary CgA level in Alzheimer's disease patients was 6.54 pmol/ml and 0.23 pmol/ml in control group. Plasma CgA in dementia patients was 85.76 ng/ml and 60.34 ng/ml in control. Statistical analysis showed significant difference level of $P \leq 0.05$. This study showed that salivary CgA levels were reduced at early stages of AD. Chromogranin A (CgA) in saliva exhibited significant reduction in immunoreactivity and to be selectively associated with prion protein deposits, CgA was only found in Amyloid beta plaques. This shows influence of constitutive amyloid protein on chromogranin secretion and role of CgA in AD neurodegenerative process. This study shows that biochemical and psychosocial stress can play major role in CgA and acts as potential biomarker for the diagnosis of AD type dementia.*

**Keywords:** Chromogranin A, Saliva, Alzheimer's disease, Dementia

## I. INTRODUCTION

Alzheimer's disease is usually characterized with symptoms like memory loss, cognitive function leading to heavy personal toll on the patients and their families with tremendous financial burden on healthcare systems[1,2]. The accuracy of currently available clinical AD diagnostic methods to predict pathologic diagnosis (in the absence of biomarker information), although looks promising in some centers but is found to be lower. A study was carried out involving research participants (n >900) and evaluated in more than 30 Alzheimer's Disease Centers in USA [3,4]. Diagnostic accuracies in primary or secondary care settings are likely to even lower. So, there is a need for the objective tests that can increase diagnostic accuracy in shorter priod of time, to aid in the design and evaluation of treatment efficacy of clinical trials for long term individual patient care [5,6,7]. It is found that psychosomatic factors and psychological stress are mostly involved in the etiology of dementia [8,9].

Chromogranin A is reported to be a acidic glycoprotein belonging to the family of regulated secretory proteins stored in dense core granules of adrenal medualla and neuroendocrine cells. It has been found that CgA is a precursor for various biologically active peptides with important roles in the regulation of neuroendocrine, metabolism and immune system [9]. Brain abrasions in Alzheimer's disease leads to dementia confined spongiform change, amyloid plaques neuronal loss, microglial activation and astrogliosis. Microglia play a important role in the prion-induced neurodegeneration [10].

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However, the intermediate molecules supporting the relationship between neurons and microglia are still unknown. The chromogranins (Cg) can trigger microglial cells leading to neurotoxic phenotype [11]. A newly measured stress related hormone Chromogranin A (CgA) is reported to have a close correlation between the dementia and healthy elder patients in saliva [12].

The biochemical markers for AD will be helpful to improve the clinical diagnostic accuracy and to increase the knowledge of pathogenesis for the disorder. The main feature of AD is degeneration of synapses. The cross sectional study of CgA has shown that it is over expressed in the saliva of type 2 diabetes, restraint stress in pigs as non-invasive biomarker [13,14]. This study emphasis the fact that psychosomatic state of a patient has impact on the production of specific proteins and epitomizing the pathogenic status of individual. This clearly indicates that limited information is present to imply the biological conceivable involvement of novel biomarker CgA in the pathogenesis of dementia. The purpose of this study is to investigate different levels of CgA in saliva and plasma in presence and absence of dementia and to assess the psychosomatic stress on the dementia type Alzheimer.

## II. Material and Methods

### 2.1. Patients

A total number of 120 patients were included in this study. 50 patients with AD type dementia and 70 healthy individuals were determined for the control group. The diagnosis of patients were carried out based on the following criteria 1) Systemic diseases 2) Administration of antidepressive within the past six months 3) History of tumor 4) Administration of drugs in the last 4-6 months 5) Chronic/acute renal failure or diseases 6) Oral chronic infectious diseases like periodontitis. Prior to the beginning of experiment 30 participants were asked to complete written questionnaire that enabled the following lifestyle pattern (habits, food intake, drugs, drinks) and Clinical Dementia Rating Scale (CDR) was used DSM-III – IV-R and to be evaluated according to the declaration of Helsinki.

### 2.2. The collection and processing of saliva samples

The study was carried out in Faculty of Medicine, Neurophysiology Laboratory, National University of Mexico, Mexico D.F. between 9 to 11 a.m. The routine recall recruitment of blood samples along with noninvasive fluids like saliva from dementia type Alzheimer patients more than 120 patients were slected for the study with wide-ranging examinations followed by MMSE, MCI [15,16]. The study was approved by the local committee of National University of Mexico and Hospital Civil on the basis of World Medical Association's declaration of Mexico as statement of ethical principles for doing medical research consisting of human subjects, research on identifiable human material and the obtained data. An ethical clearance for the above cited study was acquired from the Institutional Ethical Committee and written approval from the patients was obtained before the study was commenced [17].

### 2.3. Measurement of salivary CgA

#### 2.3.1. Saliva Sampling

The saliva sample collected from the participants in the early morning was used for the experiment [18,19,20]; the saliva samples were obtained by expectorating into polypropylene tubes. The collected salivary samples were centrifuged at 5000 rpm for 20 min and the obtained supernatant was frozen at 20°C and stored until further use.

#### 2.3.2. Plasma Sampling

The venous blood samples were collected in EDTA (1mg/ml) coated test tube using a stand venipuncture method. The plasma was separated from blood with centrifugation at 5000 rpm for 15 min. Now, the samples were stored at -20°C until it was needed for further biochemical analysis.

#### 2.3.3. Biochemical Assay

The samples collected for experiment was without oral salivary gland stimulation. The patients were instructed to salivate into the Falcon tubes (Falcon 50 ml, Germany) including 500 RL buffer with protease inhibitors (Complete cocktail tablets, Roche, Germany) inorder to preserve the proteins from degradation (Nagasawa *et al*, 1998). The samples were directly transferred into ice containers and were aliquoted to 1.5 ml Eppendorf tubes. Now it was frozen at -80°C Celsius until further

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use. Prior to the salivary CgA assay, Eppendorf tubes were centrifuged at 4°C for 12 min at 15,000 rpm. The supernatant was taken for determining the concentration of CgA present in the saliva samples (Yanaihara Institute Inc., Shizuoka, Japan). All the saliva and plasma samples were assayed in duplicates and the results were expressed in mean±SEM.

### 2.4. Data analysis

The significance of differences between data sets was analyzed by one-way ANOVA tests.

## III. RESULTS

Fig.1 shows the Demographic chart of Clinical Dementia rating Scale Score (Table.1) represent the diagnosis of AD in dementia patients for the prediction of early onset of disease precursor through physical characteristics of the symptoms which show Cognitive index like MCI, Weight Hip ratio, Body mass index and MMSE range significantly high comparable to control subjects ($p \leq 0.05$) [16]. The mean values for AD type dementia patients and controls groups are Clinical Dementia rating Scale Score (16.0-18.0 in dementia patients and 0.5-0.4 rating scale), Minimental state examination (25±0.12 in AD patients and 17±0.33 rating in controls), Mild cognitive impairment (54.5±1.23 in AD patients and 25.4±0.52 memory deficits in controls), Weight hip ratio (AD patients: 0.97±0.06 and 0.95±0.06 in controls; BMI: 27.8±3.4 in dementia and 28.5±3.8 kg/m$^2$ in control subjects which accent the status of cognitive decline. The resulted mean values were found to be significantly higher in Alzheimer type Dementia group when compared to the control cases.

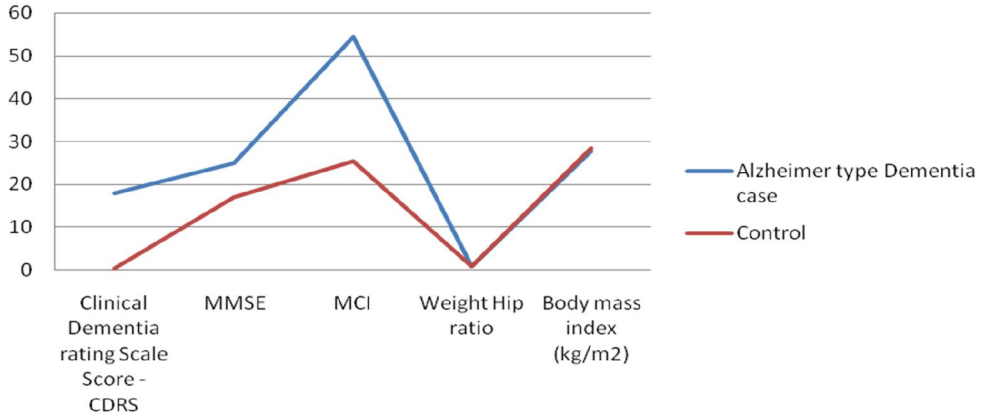


**Fig.1** Comparison of mean and standard mean value of demographic chart of psychoscomatic index in AD cases.

**Table 1** Demographic chart of AD and prevalence number in different age groups.

| Groups | Alzheimer type Dementia case | Control |
|---|---|---|
| Age (M/F) in years | 85/70 | 75/65 |
| Clinical Dementia rating Scale Score - CDRS | 16.0-18.0 (Severe dementia) | 0.5-4.0 (Normal Cognitive Impairment) |
| MMSE | 25±0.12* | 17±0.33 |
| MCI | 54.5±1.23* | 25.4±0.52 |
| Weight Hip ratio | 0.97±0.06 | 0.95±0.06 |
| Body mass index (kg/m$^2$) | 27.8±3.4 | 28.5±3.8 |

*P≤0.05 Significant difference from controls; MMSE: Minimental state examination MCI: Mild cognitive impairment

The mean values of Salivary CgA were found to be significantly higher in dementia patients than in the control group. The presence of novel biomarker CgA levels in between the cases and controls (Fig.2 and Table.2) showed significant difference statistically with the mean values 6.54±0.21 pmol/ml and 0.23±0.45 pmol/ml in saliva with P≤0.05 and in Alzheimer type Dementia case plasma levels were 85.76±0.54 ng/ml and 60.34±0.48 in control subjects. However, there was not significant difference between the CgA levels present in saliva and plasma.

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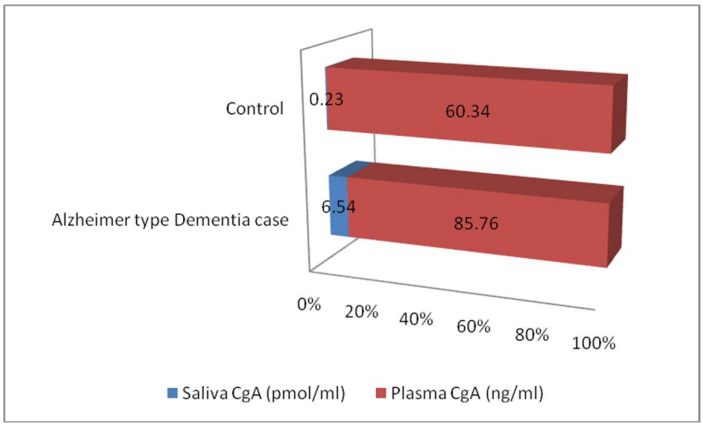


**Fig.2** shows the comparison of Salivary and Plasma Chromogranin A in Alzheimer type dementia and control cases (N=120)

**Table 2:** Comparison of CgA levels in Saliva and Plasma between dementia and control subjects (N=120)

| Groups | Saliva CgA (pmol/ml) | Plasma CgA (ng/ml) |
|---|---|---|
| Alzheimer type Dementia case | 6.54±0.21* | 85.76±0.54* |
| Control | 0.23±0.45 | 60.34±0.48 |

*P≤0.05 Significant; CgA – Chromogranin A

## IV. DISCUSSION

The clinical assessment of clinical dementia was made accorind to the rating scale scores which rates the individual into categories of cognitively normal (CDR 0), very mildly impaired (CDR 0.5), mildly demented (CDR 5-10), moderately demented (CDR 10-15), severely demented (CDR 15-20) [3]. The CDR score overall is based on the scores obtained as per the six functional domains which includes (orientation, memory, judgment and problem solving, home and hobbies, community affairs and personal care); these can be also combined into the "sum of boxes" score. The newly proposed diagnostic criteria as similar to the old criteria, clinical diagnosis of MCI is based on the impairment in one or more cognitive domains, but in the absence of dementia and maintenance of independence in daily life function. The domains where cognitive impairment occurs it has been widened to include, in addition to memory, executive functioning, language, visuospatial skills, and attentional control has been included [5]. Many interesting correlations were observed, with Tau correlating with the age of controls but not from the AD individuals, with CDR and Mini Mental State Examination (MMSE) scores, but not duration of illness and with tgender for AD group only. The meta-analysis did not show correlation between CgA, Tau and CSF Aβ42 and nor any score of severity in dementia, duration of illness or age. There has been studies reporting the negative correlation in between dementia severity and Aβ42 [21,22]. In addition to distinguishing AD from non-demented subjects. The decreased levels of CgA have been reported in addition to the distinguishing AD from non-demented subjects, which will be helpful in predicting future dementia in MCI patients. In addition decreased CgA, CSF Aβ42 levels have been observed in patients suffering from very mild dementia (MMSE score of 25-28 or CDR – 0.5) [16, 23].

vIt is well known that saliva plays a major part in the maintenance of oral mucosa and its amount and quantity can alter the oral biochemical health status. The Saliva is a noninvasive biofluid that can be easily collected, which contains locally and systematically derived markers of significant neurological diseases. There are some reports concerning with the CgA expression in saliva, halitosis and stress factors [24,9, 25]. These studies show that psychological state of patient has more impact in the production of specific proteins and mirrors the neuropathological conditions and psychosomatic state.

The collection of biofluids which include blood and saliva is found to be the most direct and convenient approach for studying biochemical changes occurring in brain. In particular fluids like CSF because of its direct contact with extracellular space of brain is considered to be attractive source for finding potential AD biomarkers. Currently, the biomarkers that is showing promise for using in AD diagnosis and prognosis include CgA, beta amyloid protein and tau. The use of CgA, Amyloid precursor protein and tau are found to be potential predictors for cognitive decline in individuals those who are suffering with very mild cognitive impairment and for predicting future dementia in the non-demented cohorts [22,21,26].

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The current study carried out is the first to show that salivary CgA level of Alzheimer demented disease subjects is found to be higher in comparision to the normal subjects. Hence, saliva is measured as the preeminent non-invasive source for biochemical study. This includes with the earlier findings, supporting that chromogranin A is acidic glycoprotein containing more than four hundred amino acids present in the secretory granules of neuroendocrine cells. During stress, catecholamines and CgA are released together into extra-cellular environment. The CgA present in salaiva can be a sensitive and promising index for AD type dementia expressed towards psychosomatic stress. Reports show that saliva is a very good source of noninvasive sampling methods and cost-effective for early onset and diagnosis of Alzheimer's disease type dementia [2].
It is prooved in the current study, that after psychocognitive improvement CgA level of AD subjects became comparable with normal subjects. CgA bein a major soluble protein present in the adrenal medullary chromaffin granules and adrenergic neurons, which is co-released along with catecholamines [15,27,3] which are considered to be a good index of sympathetic activity. Salivary CgA is also reported to be sensitive and considerable marker resulting from psychological stress, which does not respond well to physical stress [8]. The results of the experiment suggest that Salivary CgA can be a putative noninvasive biomarker for AD patients [4,3].

Age had distinct effects in the diagnostic performance of CSF AD biomarkers. As hypothesized, specificities for controls and unwavering Mild cognitive impairment decreased by means of age. In line with this, the biomarkers were relatively most useful to rule in AD in young subjects, in terms of likelihood ratios (LRs). The usefulness in absolute terms of a diagnostic test depends on disease prevalence, which increased with age in this study, causing positive predictive values to increase or remain stable (although at modest levels at the predefined cutoffs), while negative predictive values dropped slightly in the oldest. A detailed clarification of age distributions and disease prevalence is necessary to determine the true diagnostic usefulness of a biomarker test [10]. For AD, this will likely be important for all diagnostic modalities, including PET amyloid imaging, where measurements correlate tightly with CSF Aβ42. Most cutoffs at 85% sensitivity for AD dementia were similar across ages, which is in agreement with earlier reports of CSF biomarker stability during follow-up in AD. To retain a low number of false-negative AD cases age-adjusted cutoffs may not be necessary, but the presence of unadjusted cutoffs it will increase the number of "false-positives" with age, at least with the follow-up time of this study. If analyzed at specialized laboratories, minimizing measurement variability and utilizing reliable reference limits, they may also aid in dementia investigations.

Similar to other age-related disorders, including arteriosclerosis and certain cancer forms (such as prostate cancer), AD occurrence increases with aging, which lowers the difference in pathology and diagnostic methods between disease and aging with advanced age. In AD the changes in brain likely precede clinical symptoms during several years. Therefore, there are chances for a proportion of cognitively intact elderly to have preclinical AD, in presence of AD-like biomarker profile. This complicates clinical studies on very old patients with AD. The studies conducted for a period of 10–15 years need follow-up inorder to clarify the potential use of biomarkers for the clinical diagnosis of early-stage AD in elderly patients. To enable this, biomarkers need standardization to decrease interlaboratory variability, apo$\varepsilon_2$, $\varepsilon_3$ and their diagnostic performance needs to be carefully determined. Age of study subjects is an important confounding factor in AD biomarker studies, and could explain some of the variability in published diagnostic accuracies and cutoffs [21]. Great care should be taken to control for age in future studies. Studies with longer follow-up time may clarify if effects of age on diagnostic performance are caused by erroneous classifications of study subjects, or related to yet undeciphered variations in the biology of neurodegeneration and normal aging [22,21].

The (Cg) A and α-amylase have been found to be biomarkers of acute stress. Among which CgA is a 48-kDa acidic glycoprotein stored and secreted during the process of exocytosis from vesicles found in adrenal medulla and sympathetic nerves along with the catecholamines. During its secretionit correlates with the sympathoadrenal release of catecholamines. In addition, CgA is found to be localised in human submandibular glands using insitu hybridization and immunohistochemistry techniques. CgA is also reported to be present in secretory granules of ductal cells and serous by immunoelectron microscopy. It is reported that circadian rhythm for CgA in normal subjects was found to be with peak values during night time (23 hrs, mean (standard deviation) 65.4 (9.0) mg/l) and a nadir was seen in morning (8 hrs, mean (SD) 43.1 (6.6) mg/l) Giampaolo *et al* (2002). The half life of CgA for 18.4 min in blood but the correlation occurring between serum CgA and saliva so far not being reported. The presence of biomarkers in type 2 diabetes is reported [13,25,14].

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The prohormone Chromogranin A is a major ingredient of large dense core vesicles found in neurons is proteolytically processed into low molecular weight peptides in neurons before to axonal transport and it is finally released into synaptic cleft and acts as neurotransmitters. The regulation of chromogranin A was reported in AD and is found in the beta-amyloid plaques occurring in AD brain biopsies and proposed to be a potential marker for synaptic degeneration. Chromogranins are capable in activating microglial cells and metabotropic glutamate receptors. It also synergistically enhance with beta-amyloid peptides, inducing microglial neurotoxic effects and diminishing the microglial phagocytic activity in senile plaques [28]. Chromogranin A fragments acts as stimulators for the development of senile plaque development, neuronal toxicity and its changes in concentration during AD-treatments can be used as surrogate markers [8]. The disturbances occurring during maturation and degradation of synaptic neuropeptides is one of the reason for cognitive malfunction and neuronal loss. The abnormal proteolysis caused by enhanced protease inhibitor activities because of the synaptic peptides found in this study of AD patients plays a major role in cognitive impairment and AD pathology before the formation of tangle and plaque. Therefore, proteases like prohormone convertases can find its way as new drug targets for the development of AD treatments. Thus the identification of new biomarkers, study of established markers of AD like fragments of amyloid-beta peptides and AD-markers like the APLP1 fragments points is found to have higher potential.

## V. CONCLUSION

The present results presented here describe a method for accurate determination of salivary chromogranin A. The results show that salivary chromogranin A is a stress hormone protein produced by hypothalamopituitary axis and adrenal medulla of the body. It is found that as fundamental molecule it is being constantly produced throughout the body. The secretion of salivary chromogranin A in saliva is a manifestation of this production. Measurement of salivary chromogranin A levels in a series of volunteers demonstrated some remarkable results. Many studies have suggested that saliva proteins contain biomarkers for the neurodegenerative diseases. Statistically significant protein differences have been found in AD saliva, whereas large-scale studies needs to be conducted as to confirm, whether saliva can be used in clinical test for neurodegenerative disease.

## ACKNOWLEDGMENT

The author wishes to thank Prof. R G Guzman, National university of Mexico, Mexico D.F for providing facility to carry out this study through DGAPA BECAS postdoctoral research Program. The facility availed from DST-FIST, Government of India GA acknowledges with thanks to UGC, New Delhi for the award of UGC-BSR Faculty Fellow [F.No.18-1/2011 (BSR) dt. 4.1.2017].

## REFERENCES

**[1].** H. Haririan, K. Bertl, M. Laky, W-D. Rausch, M. Bottcher, M. Matejka, O. Andrukhov, X. Rausch-Fan, *J Periodontol*, 2012, 83, 1314-1321.
**[2].** R. Farah, H. Haraty, Z. Salame, Y. Fares, N.S. Sadier, *Biomedical Journal*, 2018, 41, 63-87.
**[3].** G.M. McKhann, D.S. Knopman, H. Chertkow, *Alzheimers Dement*, 2011, 7, 263-269.
**[4].** G. De Meyer, F. Shapiro, H. Vanderstichele, *Arch Neurol*., 2010, 67, 949-956.
**[5].** P.J.Visser, F. Verhey, D.L. Knol, *Lancet Neurol*., 2009, 8, 619-627.
**[6].** G.M. Savva, S.B. Wharton, P.G. Ince, G. Forster, F.E. Matthews, C. Brayne, *N Engl J Med*., 2009, 3, 2302-2309.
**[7].** S. Rolstad, A. Nordlund, C. Eckerstrom, M.H. Gustavsson, H. Zetterberg, A. Wallin A, *Dement Geriatr Cogn Disord*., 2009, 27, 194-200.
**[8].** N.Mattsson, P.Johansson, O.Hansson, A.Wallin, H.Zetterberg, *Alzheimer's & Dementia*, 2010, 6, S511.
**[9].** A.P. Reshma, R. Arunachalam, J.K. Pillai, S.B. Kurra, V.K. Varkey, M.J. Prince, *J Ind Soc Periodontol*., 2013, 17, 214-218.
**[10].** D.A. Bennett, *Neurology*, 2013, 80, S690.
**[11].** F.Verde, P.Steinacker, P.Oeckl, J.H.Weishaup, M. Otto, *Neurobiology of Aging*, 2018, 67, 21-22.
**[12].** A. Yoto, S. Murao, Y. Nakamura, H. Yokogoshi, *J Physiol Anthropol*., 2014, 33, 20-20.
**[13].** M. Soell, A. Feki, M. Hannig, H. Sano, M. Pinget, D. Selimovic, *Bosn J Basic Med Sci*., 2010, 10, 2-8.
**[14].** Y. Huang, Z. Liu, W. Liu, C. Yin, X. Yang, *Research in Veterinary Science*, 2017, 114, 27-30.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 2, March 2022
Impact Factor: 6.252

**[15].** Y.Kanamaru, A. Kikukawa, K. Shimamura, *Stress*, 2006, 9, 127-131.
**[16].** T. Tombaugh, I. McDowell, B. Kristjansson, A. Hubley, *Psychological Assessment*, 1996, 8, 48–59.
**[17].** World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013: 27; 310(20), 2191-4. doi: 10.1001/jama.2013.281053
**[18].** M. Navazesh, *J Calif Dent Assoc*., 2011, 39, 626-8.
**[19].** S. Alagendran, G. Saibaba, S. Muthukumar, R. Rajkumar, R.G. Guzman, G. Archunan, *Ind J Dental Res*., 2013, 24, 157-163.
**[20].** S. Alagendran, G. Archunan, S. Velayutha Prabhu, B. Enrique-A Orozco, Rosalinda Guevara Guzman Dra, *Ind J Dental Res*., 2010, 21, 165-168.
**[21].** M. Shi, Y.T. Sui, E.R. Peskind, et al., *J Alzheimers Dis.,* 2011, 27, 299-305.
**[22].** F. Bermejo-Pareja, D. Antequera, T. Vargas et al., *BMC Neurol.,* 2010, 10, S108.
**[23].** M. Toda, R. Den, M. Hasegawa-Ohira, K. Morimoto, *Complementary Therapies in Medicine*, 2013, 21, 29-34
**[24].** S. Gallina, M. Di Mauro, M.A. D'Amico, E. D'Angelo, A. Sablone, A. Di Fonso, A. Bascelli, P. Izzicupo, A. Di Baldassarre, *Clin Endocrinol*., 2011, 75, 747-752.
**[25].** E.M. Kogawa, D.C. Grisi, D.P. Falcao, I.A. Amorim, T.M.B. Rezende, I.C.R. da Silva, O.N. Silva, O.L. Franco, R.F.B. de Amorim, *Arch Oral Biol.,* 2016, 62, 10-19.
**[26].** M. Lee, J.P. Guo, K. Kennedy, G. Edith, E.G. McGeer, P.L. McGeer, *J Alzheimers Dis.,* 2016, 1-8.
**[27].** R. Den, M. Toda, S. Nagasawa, K. Kitamura, K. Morimoto, *Biomed Res*., 2007, 28, 57-60.
**[28].** A. Giuffrida, M. Vanoli, M. Martino,V. Lenti, A. Di Sabatino, *Digestive And Liver Disease*, 2018, 50, E140-E141.