Iga and Alpha-Amylase: New Targets for Well-Being in Alzheimer's Disease Patients, a Pilot Study with Music Therapy

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Abstract

Well-being plays a significant role in Alzheimer's disease (AD) and music therapy can bring about an increased perception of well-being in these patients. The aim of this study is to determine the relationship between the improvement in the emotional perception and the physiological parameters (immunoglobulin A (IgA) and alpha-amylase). A prospective, mixed analytical and quasi-experimental study was carried out, involving eight music therapy sessions over a two-month period in patients with mild AD. The final sample for this pilot study contained 25 patients from Valencia (Valencia Association of Relatives of Alzheimer's Patients (Spain)) and the experiment was performed between the months of October and November of 2015.

The ELISA technique was used to quantify salivary IgA and alpha-amylase before and after the sessions. Mood questionnaires were also used to analyze mood state happiness. Salivary IgA and alpha-amylase were found to be correlated and to increase after music therapy although the differences were not significant. The happiness scores were seen to increase significantly, but showed a non-significant association with IgA production. Therefore, IgA and alpha amylase could be used as possible biomarkers of increases in well-being in AD patients, although more studies are needed to confirm these results.

Keywords

Alzheimer's, Immunoglobulin A, Alpha-amylase, Well-being, Music therapy

Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder amongst the elderly. In occidental societies the prevalence is high, with several social, behavioral, mental and health consequences [1]. However, it has no cure and nowadays, the treatments for Alzheimer's disease only offers limited symptomatic relief. Therefore, more knowledge about the factors associated with this disorder and effective therapies agents are urgently needed [2].

In these patients, different mood impairments and changes in their well-being have been shown [3]. Well-being is construed as a combination of positive emotions, engagement, meaningful

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relationships and a sense of accomplishment [4] thus reflecting positive aspects of mental health. It is known that AD patients have emotional problems and that it can induce physiological consequences. For example, immunoglobulin A (IgA) being an important biomarker of the immune response system. Its production has been shown to be higher in situations of improved well-being [5]. In this regard, it has also been known for some time that cheerfulness and positive emotions are accompanied by an increase in salivary IgA levels [6].

In addition, noradrenalin is the sympathetic nervous system's main neurotransmitter. Also, it provides information about the patient's emotional state. Increases in endogenous noradrenalin are recorded when subjects receive emotional stimuli, and are significantly correlated to the suppression of these stimuli but not to the suppression of emotionally neutral stimuli [7]. Activation of the adrenergic system can be evaluated by quantifying alpha-amylase in parotid gland saliva [8] thus becoming another important biomarker of the emotional state. Saliva seems a good option to measure IgA and alpha-amylase in the same sample because it is easy to obtain and non-invasive, as well as being very representative of the physiological condition due to the significant vascularization of the oral cavity and the proportionality between the composition of blood and saliva.

On the other hand, it has been demonstrated that environmental factors can be involved in the appearance and progression of different disorders [9]. Studies have observed that negative context or stress situation can induce mood and cognitive impairments [10] and that a negative environment can promote the acquisition and maintenance of different chronic mental disorders, such as, drug addiction [11]. Similar results have been demonstrated in AD using a positive context. In patients with mild AD, it has been observed that an enjoyable environment using nonpharmacological therapies (NDTs) improve neuroplasticity [12,13], and produce improved psychological and physical well-being, optimize the quality of life and encourage social integration. Several nonpharmacologic therapies have been explored and showed improvement in patients with dementia, these include art therapy [14] and doll therapy [15] which theoretically fulfils patients' attachment needs. Between theses nonpharmacological therapies, also music therapy has demonstrated benefits in the patients' quality of life (i.e. decreasing anxiety

and depression). It has been shown to have benefits on the patients' quality of life, preserving the skills of expression and socialization, improving anxiety, depression, irritability and social isolation [16]. Moreover, this therapy is being used in the field of dementia because it is a non-invasive technique and does not need complex cognitive understanding [17].

For these reasons, the aims of this study were firstly to evaluate the role of IgA and alpha-amylase as possible useful biomarkers after applying a positive environment based on music therapy in the AD patients and secondly, to determine the possible relationship between the two parameters (physiological and emotional).

Methods

■ Design

A prospective, mixed analytical and quasi-experimental study design was used.

Participants

The selection criteria was: patients over 65 years of age without an aversion to music therapy, who regularly visit the Valencia Association of Relatives of Alzheimer's Patients (Spain) (*Asociación de Familiares de Alzheimer de Valencia*, AFAV), using a Mini-Mental State Examination (MMSE) score ranging from 18-23 and obtaining legal consent from each patient's guardians. The MMSE is a commonly used method to study cognitive impairment and to assess the outcome of patients with these symptoms through different questions and tasks that the patients are asked. The evaluation system consists in increasing the punctuation when the patient responds correctly, allowing us to assess different aspects like orientation, attention, concentration, memory or language [18]. In previous works, the test had showed good sensitivity and specificity levels, 0,87 y 0,82 respectively [19]. The exclusion criteria were patients that refused to participate in the study, patients with any other mental or physical illnesses and patients taking drugs that could interfere with the results.

The final sample for this study contained 25 patients (n=25) with a mean age of 73.27 ± 4.8 years and without discrimination between sexes. All the participants had a high level of education (they had completed university studies) and they had a high socioeconomic status **(Table 1)**.

Table 1: Information of the study participants.					
Group Music Therapy	Time of Day	Day of Week	Time of Therapy	Demographic Information	Severity of Alzheimer´S Disease
GROUP 1 (N=12)	9:00	TUESDAY	60 MIN DURING 2 MONTH	Mean age of 72.61 ± 5.2 Non discrimitacion of sexe High level of education High socioeconomic status	Mild State
GROUP 2 (N=13)	9:00	TUESDAY	60 MIN DURING 2 MONTH	Mean age of 73.93 ± 4.4 Non discrimitacion of sexe High level of education High socioeconomic status	Mild State

All the patients that participated in this study took similar drugs, in order to reduce external variables. These drugs were inhibitors of acetylcholinesterase and anxiolytics of the group of benzodiazepines. These drugs were prescribed and administered by the doctors of the association who collaborated in the study, keeping the same doses and dosage scrupulously throughout the intervention. "Moreover, the legal representative of the patients committed, before the start of the trial, to not administer during the same period any type of pharmacological or nutritional supplement that could change the mood or cognition of the patients".

■ Data collection

The 25 subjects were randomly chosen by means of a Microsoft Excel 2010 spreadsheet into two groups of 12-13 people in order to improve the work of the music therapist, to increase the effectiveness of the intervention with reduced groups and reduce the anxiety levels of the patients working in reduced groups. The treatment was implanted at the same time (9:00 a.m.) and on the same day of the week (Tuesday) during the two-month period, lasting 60 minutes per session in a relaxed environment and with a stable temperature of 22ºC, involving two activities (30 minutes per activity). The place of sampling was the Association of Alzheimer's Patients in Valencia (Spain). It is an innovation center in Valencia with adapted rooms for the patients. All of the patients attended all of the sessions.

1. A welcome song designed to activate the cognitive area, improving recent memory, remembering the names of classmates, the lyrics of the song, visual agnosia with the recognition of faces, attention with concentration of the musical task, and orientation with the recognition of band mates and recognition of the day of the week (day session). Social-emotional stimulation to improve self-esteem from a successful experience, and participation in vocal group activity at verbal level, interacting with peers.

2. The theme song related to flowers, with the aim of improving subject cognitive level and memory, remembering the lyrics of the songs and the songs worked in the session; the language for singing the lyrics of the songs and saying the name of the flowers and / or plants named in the songs; attention focusing on the musical task; temporal orientation linking flowers with the spring season. Social-emotional stimulation to improve self-esteem from a successful experience, and participation in vocal group activity at verbal level, interacting with peers. Expression of feelings and opinions and / or musical preferences. Stimulation of respiratory function (singing) at physical-motor level.

The center's psychologists gathered the information from the mood questionnaires before and after the interventions, with each psychologist repeating with the same patient before and after the sessions.

The nurses were blinded to the patient group assignment. They also collected saliva samples from each patient (before and after each session) for a period of two minutes (2 ml in autoclave-sterilized glass tubes). The samples were stored at -80ºC, then after thawing and gentle centrifugation for 30 seconds, the IgA and alpha-amylase levels were determined using a solid-phase, competitive chemiluminescent enzyme immunoassay (ELISA) (Immulite, Siemens).

■ Validity and reliability

The patients' mood was assessed using standardized interviews, specifically a version of the mood questionnaire adapted by Górriz, Prado-Gascó, Villanueva, Ordóñez and González. The election of the test was based on the adequate levels of reliability showed [20] contemplating four mood states: happiness, anger, sadness and fear, measured by 16 items scored on a three-point scale (1 = never, 2

= sometimes, 3 = often). We extracted the answers related to the mood state of happiness (specifically four items: I feel content, I feel cheerful, I feel very happy, I feel elated), since this was taken to be the mood state that best represents perceived well-being. Accordingly, the sum of the items' scores ranged from 0-12 points, and in all dimensions the higher scores were indicative of greater happiness.

■ Ethical considerations

The study was approved by Valencia University's Clinical Research Ethics Committee and we obtained legal signed consent from each patient's guardians. We considered all the basic principles of biomedical research described in the Declaration of Helsinki. The procedure number provided by the ethical committee for human research of the experimental research ethics commission of the University of Valencia was H1435215051072.

■ Data analysis

Data were analyzed with Shapiro-Wilk and Levene's tests. Significance was explored based on the Student t-test, and correlations were evaluated with the Pearson correlation coefficient. The tool/programme used to conduct the statistical analyses was the SPSS version 24.

Results

■ A.-Levels of IgA and alpha-amylase

The Student's t-test showed that the IgA levels increased in 15 of the patients after therapy (60%), with a mean variation in IgA concentration of 16.9 ± 86.3 mg/dl versus the levels before therapy; this change in IgA concentration was not statistically significant, however (p=0.336). Likewise, alpha-amylase increased in 14 patients (56%), with a mean variation of 152.0 ± 1261 U/l versus the levels before therapy, though here again statistical significance was not reached (p=0.504) **(Table 2)**.

The Pearson test showed a positive correlation between the alpha-amylase and IgA values produced by the patients following the music therapy (r = 0.778, with R2 = 0.606) **(Figure 1A)**.

■ Relationship between well-being and IgA levels

The Student's t-test showed that the differences in happiness score before and after music therapy were statistically significant (p<0.001) **(Table 2)**. The Pearson coefficient showed that the greater the increase in IgA after therapy, the higher the patient's perceived happiness score, although a non-significant positive correlation was established between the changes in IgA and happiness (r = 0.25, with R^2 = 0.062) **(Figure 1B)**.

Discussion

After analyzing the values of IgA and alpha-amylase, an increase (although not significant) is seen in the production of these two molecules. This result agrees with others obtained in our laboratory, where a decrease in stress was observed (measured by a drop in cortisol) after applying NDTs based on ICTs with Alzheimer's patients [21] and an increase in IgA following NDTs applied to another type of patient. However, our findings differ from those of other authors who have reported decreases in the levels of noradrenalin after music therapy given to elderly people without AD [22]. Such differences could be due to the fact that music therapy in this work was applied over a very long period of time in geriatric patients with cerebrovascular diseases, seeking functional cardiac improvements by lowering the adrenergic tone, not one-off emotional improvements as in our case. Moreover, we observed a relationship between the production of both biomarkers that had not been observed until then in Alzheimer's patients, but had been seen in obese children with high arterial pressure, though in the opposite sense [23] maybe due to the neuroendocrine changes associated with this disorder.

Furthermore, in our study an increase in the perception of well-being is also observed, similar to the results obtained [16] after music

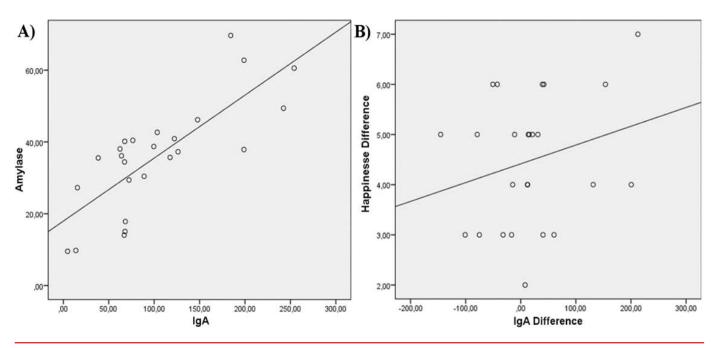| Table 2: Mean values of IgA and alpha-amylase in saliva, and perception of wellbeing (happiness), with differences of means, confidence intervals and p-values, before and after music therapy.						
	n	Before therapy	After therapy	Mean difference	95%CI	p-value
IgA (mg/dl)	25	102.8 ± 68.0	119.7 ± 88.2	16.95	-18.70 to 52.61	0.336
Amylase (U/l)	25	1521.0 ± 1181.9	1673.1 ± 1250.1	2.04	-4.16 to 8.25	0.504
Happiness	25	7 ± 1.32	11.48 ± 1.23***	4.48	3.95 to 5.01	0.000
* Significantly different p<0,05; ** Significantly different p<0,01; *** Significantly different p<0,001						

Figure 1: Correlations between: IgA and alpha-amylase after music therapy. Differences in happiness score (Happiness difference) and IgA levels (IgA Difference) after music therapy.

therapy. Moreover, other authors showed that the patients with the highest happiness scores also had the highest salivary IgA levels [24] but no previous work has studied the correlation between physiological and emotional parameters in Alzheimer's patients.

Thus, our results support the idea that music therapy improves the happiness levels in mild AD patients observing statistically significant differences before and after this type of therapy. However, the conclusions in our study about the role of IgA and alpha-amylase in AD patients' and the relationship with positive emotional state are more restricted because we observed a tendency but we can't confirm our results. Music therapy could improve the levels of these biomarkers and consequently the perception of emotional well-being in these patients although our results showed that the change in both alpha-amylase and IgA after music did not achieve statistical significance. Moreover, IgA and happiness scores were not significantly correlated either. For this reason, the role of both biomarkers as a potential diagnostic tool for increased knowledge in well-being in AD patients is limited and it is only a pilot study.

In fact, we would like to remark that our pilot study has some limitations, like the small sample used and that it is necessary to confirm these results with a group with no therapy at the same time. Also, a greater number of measures would be necessary in time to deepen the evolution of the changes observed. For the moment, our conclusions only showed a tendency but we can´t draw general conclusions from our results. We suggest that similar studies are needed in order to advance in the knowledge of the efficacy of this therapy and biomarkers of well-being in AD.

Author contribution (Roles)

Jose Enrique de la Rubia Orti: Design and drafting of procedure.

María Pilar García Pardo: Drafting and interpretation of results.

Pilar Pérez Ros: Drafting the introduction and discussion sections.

Mariano Julián Rochina: Stadistical interpretation of results

Sandra Sancho Castillo: Laboratory task and data collection.

Research   José Enrique de la Rubia Ortí

Conflict of Interest

Funding Statement

Ethical Approval

The study was approved by Valencia University's Clinical Research Ethics Committee and we obtained legal signed consent from each patient's guardians. We considered all the basic principles of biomedical research described in the Declaration of Helsinki.

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