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Original Article
Artigo Original

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Occurrence of psychiatric conditions, use of psychotropic medications and its relationship with postural balance in subjects with dizziness

Ocorrência de condições psiquiátricas, uso de psicotrópicos e sua relação com o equilíbrio postural em sujeitos com tontura

Keywords

Dizziness
Vestibular Function Tests
Postural Balance
Mood Disorders
Psychotropic Drugs

ABSTRACT

Purpose: to analyze the occurrence of psychiatric diagnosis and the use of psychotropics medications in subjects with vestibular complaints and to relate the presence of these conditions to the results of vestibulometry. **Methods:** quantitative, observational, cross-sectional study with 131 patients, treated in a university hospital. They were submitted to anamnesis, visual inspection of the external ear canal, static and dynamic balance tests, Foam laser dynamic posturography and Computerized Vectoelectronystagmography. **Results:** sample composed of 109 women and 22 men, with average age of 55 years and nine months. The most common type of dizziness was vertigo, with the presence of neurovegetative signals. A significant percentage of psychiatric complaint/diagnosis was observed, as well as the use of psychotropic medications, mainly serotonin uptake inhibitors, followed by benzodiazepines. There was a relation between the presence of psychiatric complaints with the female gender, alterations of the static balance and alterations in the Sensorial Organization Test positions III and VI. In the Vectoelectronystagmography, there was a relation between age and the presence of spontaneous nystagmus. **Conclusion:** There was a high occurrence of psychiatric complaint/diagnosis among patients with dizziness, with use of psychotropic medications substantially greater than the general population. The evaluation of postural balance revealed an association between anxiety/depression and alterations visual overload positions in the foam laser dynamic posturography. However, no relationship was found between these conditions and alterations in the Vectoelectronystagmography tests.

Descritores

Tontura
Testes de Função Vestibular
Equilíbrio Postural
Transtornos do Humor
Psicotrópicos

RESUMO

Objetivo: analisar a ocorrência do diagnóstico psiquiátrico e o uso de psicotrópicos em sujeitos com queixas vestibulares e relacionar a presença dessas condições aos resultados da vestibulometria. **Método:** estudo quantitativo, observacional, transversal, com 131 pacientes, atendidos em um hospital universitário. Foram submetidos à anamnese, inspeção visual do meato acústico externo, provas de equilíbrio estático e dinâmico, Posturografia dinâmica *foam laser* e vectoeletronistagmografia computadorizada. **Resultados:** amostra composta por 109 mulheres e 22 homens, com média de idade de 55 anos e nove meses. O tipo de tontura mais frequente foi vertigem, com presença de sintomas neurovegetativos. Observou-se expressiva porcentagem de queixa/diagnóstico psiquiátrico, bem como uso de psicotrópicos, sendo principalmente inibidores seletivos da recaptação da serotonina, seguidos dos benzodiazepínicos. Houve relação entre a presença de condições psiquiátricas e mulheres, alterações do equilíbrio estático e alterações nas posições III e VI do Teste de Organização Sensorial. Na vectoeletronistagmografia, houve relação entre a idade e a presença de nistagmo espontâneo de olhos fechados. **Conclusão:** Constatou-se alta ocorrência de condições psiquiátricas entre pacientes com tontura, com uso de psicotrópicos maior que na população geral. Destaca-se a associação entre ansiedade/depressão e alterações nas posições de sobrecarga visual da posturografia dinâmica *foam laser*. No entanto, não foi observada relação entre essas condições e alterações nas provas da vectoeletronistagmografia.

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INTRODUCTION

The vestibular system has three main functions: to maintain balance through vestibulospinal reflexes, to stabilize vision through Vestibulo-Ocular Reflex (VOR) and to contribute to spatial perception and orientation[1]. Sensory disintegration may lead to dizziness, a common symptom in medical practice, reported by the general population, resulting from vestibular and non-vestibular dysfunctions[2]. It has a varied etiology, being peripheral or central, with great variation in the duration of symptoms and the circumstances that produce them[1].

Vestibular symptoms can be classified as 1) vertigo, 2) orthostatic dizziness (or produced by sounds and images), 3) vestibulovisual symptoms, such as oscillopsia and 4) postural symptoms, such as imbalance and instability[3]. Vertigo usually has a vestibular origin, while the second may reflect a fall in cerebral blood flow; vestibulovisual symptoms involve the vestibulo-ocular reflex or ocular motility; postural symptoms may indicate a neurological disorder[4].

Those symptoms that do not fit into any of the above categories are still considered. They can be called only by dizziness, it encompasses cases of multifactorial etiology, such as metabolic and psychiatric[4]. According to Eckhardt-Henn[5], in 55% of cases of dizziness, psychiatric disorders seem to present an important influence on the course of the disease, with organic origin being the smallest part compared to psychogenic causes.

The association between mood disorders and dizziness is well known in the literature[6]. Both conditions may lead to nonspecific complaints, such as changes in sleep patterns, increased muscle tone, irritability, fear, restlessness and worry. Some authors explain part of this relation to the sharing of neural pathways[7].

The use of psychotropic medications as a treatment for mood disorders has increased over the last years. Studies carried out in Europe in the 2000s indicate that its prevalence over a period of 12 months was 19.2% in France, 15.5% in Spain, 13.7% in Italy, 13.2% in Belgium, 7.4% in Ireland and 5.9% in Germany[8]. In Brazil, between 2008 and 2009, the prevalence of psychotropic acquisition was 5.2%[9].

Patients with depression may have somatic symptoms, including dizziness, and in these cases, antivertiginosis is replaced by psychotropic medications[10]. Among the population of individuals with altered balance, the use of psychoactive drugs is recurrent, however, there is no extensive exploitation of these factors in the literature. Increased levels of distress in patients with chronic symptoms suggest that their emotional state contributes to prolonging the symptoms of acute phase dizziness[11].

The relationship between mood disorders and dizziness still lacks explanations from certain perspectives, for example, *what aspects of postural balance maintenance does the psychiatric condition affect*?. Thus, this study aims to analyze the occurrence of psychiatric diagnosis and the use of psychotropics in subjects with vestibular complaints and, later, relate these conditions to the results of vestibulometry.

METHODS

This is an observational, cross-sectional, retrospective study of quantitative analysis, approved by the Research Ethics Committee (REC), under CAAE number 16728013.0.0000.5346.

The studied variables were obtained by reviewing a database composed of 342 medical records of patients with dizziness, both men and women, referred to the Otoneurology Outpatient Clinic - Equilibrium Sector - from a university hospital in the period from 2012 to 2016. The criteria inclusion were: to have as main complaint dizziness (vestibular or extravestibular origin); to be 18 years of age or over; to complete the battery of tests proposed in this study; to have otorhinolaryngological evaluation; and, for the subjects with psychosocial profile, the psychiatric evaluation.

The exclusion criteria adopted were: to have a history of middle or inner ear infections, neurological or visual pathology, to be oncological treatment or frequent use of alcohol; not to have data in the chart on psychiatric evaluation for those with a psychosocial profile indicating psychopathology. Based on these criteria, 131 exams were selected to compose the sample. For this study to be part of a mother project, all the subjects involved previously signed the Free and Informed Consent Term.

After the selection, the exams were separated into two groups: subjects without psychiatric diagnosis or any history of mood disorders and the group of subjects with psychiatric diagnosis (with and without the use of psychotropic medications).

Procedures

The days and times of the evaluations were pre-scheduled; the subjects who participated in the research were given an indispensable preliminary preparation for the examination: 1) to abstain from the use of labyrinth stimulants (caffeine, chocolate, alcoholic beverages) and/or narcotics for a period of 24 hours before the examination; 2) to abstain from depressant drugs of the Central Nervous System for 48 hours; 3) to perform a light meal three hours before the exam, avoiding fasting; 4) to avoid the use of contact lenses; 5) to avoid makeup and creams to facilitate electrical conductivity[12].

All were submitted to structured anamnesis to obtain the previous and current clinical history, with questions about presence and characteristics of dizziness, otological health, general health history and drug treatments, as well as possible comorbidities and previous therapeutic treatments.

Visual inspection of the external auditory meatus and the tympanic membrane with the Heidji-type otoscope was performed to verify the integrity of the structures and impediments that would make it impossible to perform the caloric test on water in the Computerized Vectoelectronystamography (VENG).

Static and dynamic balance tests

The tests of static and dynamic balance were: 1) Romberg test: patient in orthostatic position with arms along body; 2) Babinski-Weil test: to walk on a course of approximately 1.5 m (five steps forward and five steps back); 4) Unterberger

test: to march by raising the knees approximately 45 without moving, performing 60 steps (1/s) with the arms extended forward. All the tests were performed with eyes open and then closed, lasting 60 seconds. For greater reliability of the results, the evaluations were performed in the room with controlled light and sound sources, avoiding orientation[12].

Posturographic evaluation

Foam-Laser Dinamic Posturography[13] was performed by analyzing the results of the six positions of the Sensory Organization Test (SOT) (Figure 1), in which the anteroposterior deviations were verified by calculating the measurements of each SOT by means of Excel program. Visual, somatosensory and vestibular function preferences were analyzed according to the means of the SOT according to the following formulas: Somatosensory function: SOT II/SOT I; Visual function: SOT IV/ SOT I; Vestibular function: SOT V/SOT I; Balance index: (SOT III + SOT VI) / (SOT II + SOT IV). The normal values for SOT are: SOT I (90%), SOT II (83%), SOT III (82%), SOT IV (79%), SOT V (60%), SOT VI(54%).

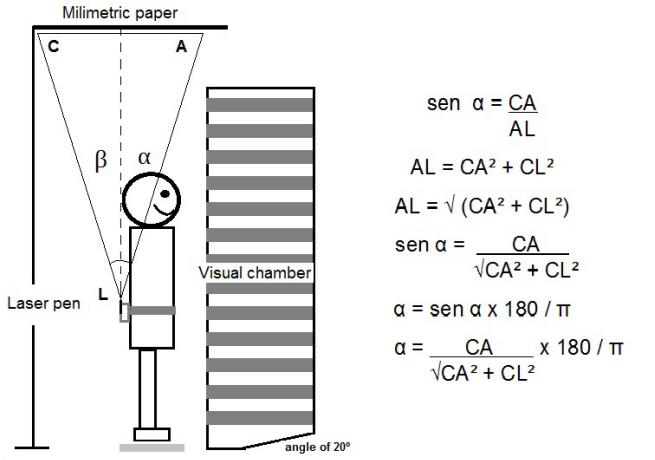
Computerized Vectoelectronystamography (VENG)

The Computerized Vectoelectronystamography system (VENG), of the brand Contronic, model SCV, version 5.0, was used in room with electrical grounding to avoid the interference in the electric current.

The leds bar was positioned 1 m away from the assessed subject, towards the midline of the eyes, for standardization purposes. The examination was performed in a quiet room and controlled light. Initially, the subject's skin was cleaned, followed by the attachment of four electrodes, with electrolytic paste and adhesive tape (micropore), periocular region on the right and left lateral corners, another on the frontal region (ground) and the last, two centimeters above the glabella (active), allowing the registration of the horizontal, vertical and oblique movements of the eyes.

Subsequently, the oculomotor tests were performed:1) Calibration of horizontal and vertical ocular movements: displacement of two alternating light points at 10 of angular deviation of the eyes, at a speed of 10 mm/s; 2) Spontaneous Nystagmus (SP) with open eyes, and then closed; 3) Right, left, inferior and superior Semispontaneous nystagmus (SPN); 4) pendular tracking: visual accompaniment of the pendular movement of a luminous point, resulting in a sinusoidal curve of the ocular movement; 5) Optokinetic Nystagmus (ON): visual accompaniment of moving dots to the right and then to the left.

Tests also were performed in the Barany chair: 1) Rotatory Chair Test (RCT), to evaluate the synergism of the lateral semicircular canals, placing the head in the 30° anterior position, with closed eyes, during the pendulum of the chair in the clockwise and counterclockwise; 2) Caloric test, with water stimulus (240 ml), for 40 seconds at temperatures of 44 °C and 30 °C, in the following sequence: 44 °C - right ear (OD), 44 °C - left ear (OE), 30 °C - OE and 30 °C - OD; with interval of three minutes between the tests[12]. The subjects were positioned so that they remained in dorsal decubitus position, at a 30º inclination in relation to the horizontal plane (I Brunnings position) (lateral canals upright)[12].



$$\text{sen } \alpha = \frac{CA}{AL}$$

$$AL = CA^2 + CL^2$$

$$AL = \sqrt{(CA^2 + CL^2)}$$

$$\text{sen } \alpha = \frac{CA}{\sqrt{CA^2 + CL^2}}$$

$$\alpha = \text{sen } \alpha \times 180 / \pi$$

$$\alpha = \frac{CA}{\sqrt{CA^2 + CL^2}} \times 180 / \pi$$

I. The calculation of the anterior balance angle is performed by the formula's application to the 90º angle. The same value is used to calculate the β angle corresponding to the posterior balance.
II. Both angles (α–β) are added to represent the total of oscillations of the gravity center.
III. The formula is used to indicate the oscillations which agree with the counting format of the SOT balance (100% = o balance – 0% = balance decrease or maxinal swerve 12,5º).

$$\text{Balance (\%)} = 100 - \{100 \times (\alpha+\beta) / 12,5\}$$

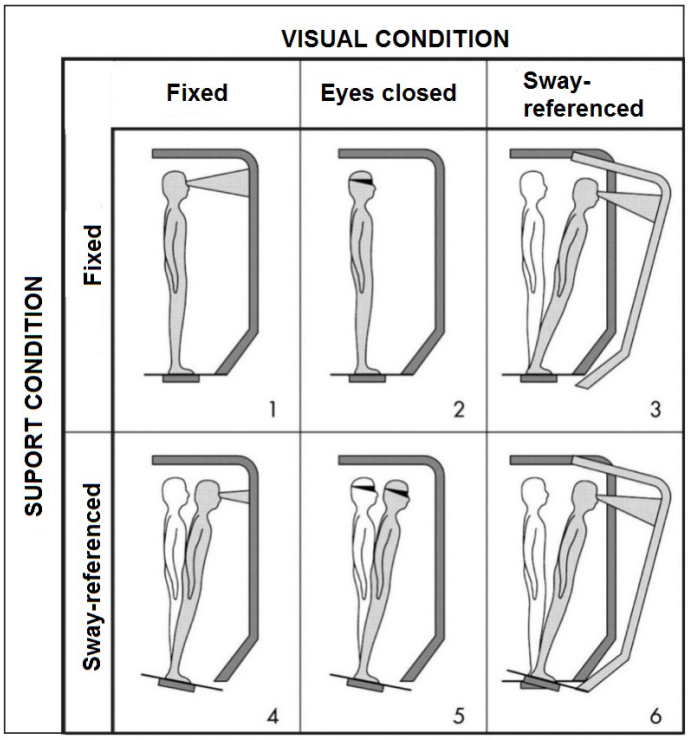


Figure 1. Mathematical formula for the calculation of the body oscillation angle proposed by the foam laser dynamic laser posturography technique and the six positions of the Sensorial Organization Test[14]

For the recording of post-caloric nystagmus, the subjects remained with their eyes closed and under mental task, in order to maximize the responses of the VOR. After obtaining the tracing, they were instructed to open their eyes and fix them at a point in front of them to observe the eye fixation[12].

Normal values were: calibration of regular horizontal and vertical ocular movements; open eyes spontaneous nystagmus absent and eyes closed with Slow Component Angle Velocity (SCAV) up to 7º/s; vertical and horizontal semispontaneous nystagmus absent, horizontal and vertical pendular tracing (type I or II); optokinetic nystagmus (up to 20%); 30% RCT and 30% caloric test for labyrinth or directional predominance of nystagmus; SCAV less than 3º/s for hyporeflexia and more than 50º/ s for hyperreflexia[12].

Data analysis

The data were stored in an Excel spreadsheet. A descriptive analysis of the qualitative and quantitative data was carried out, following normality through the proposed values for Foam Laser Dynamic Posturography and VENG. The comparative inferential analysis among the groups with or without psychiatric diagnosis and, subsequently, with or without the use of psychotropic medications, both related to the results of the evaluations, was performed using the nonparametric Pearson's Chi-Square Test or Fisher's Exact Test when $n < 5$. For this, the statistical software STATISTICA 9.1 was used, with a significance level of 5% ($\alpha = 0.05$).

RESULTS

The sample consisted of 109 (83.21%) women and 22 (16.79%) men, with a mean age of 55 years and nine of months (+15.67), ranging from 18 to 85 years.

The most frequent type of dizziness was vertigo (55.73%), with presence of neurovegetative symptoms (53.44%). An expressive percentage of psychiatric diagnosis (74-56.49%) classified as pathological anxiety and depression (Gt + d) was observed. Some subjects received multiple diagnoses, such as anxiety + bipolar disorder, depression + personality disorders, however all those considered in the psychiatric diagnosis group presented with anxiety or depression.

In the general sample, the use of psychotropic medications was identified in 51 subjects - 38.93% - (Gt+p), of whom only one was on psychotropic treatment specifically for dizziness without a psychiatric diagnosis. All patients taking psychotropic medications used at least 30 days before otoneurological evaluation. There was a relationship between the psychiatric diagnosis and the women, but not regarding the use of psychotropic medications (Table 1). The most commonly used types of psychotropics were Selective Serotonin Reuptake Inhibitors (SSRIs), followed by benzodiazepines, with the combined use of two or more types (Figure 2).

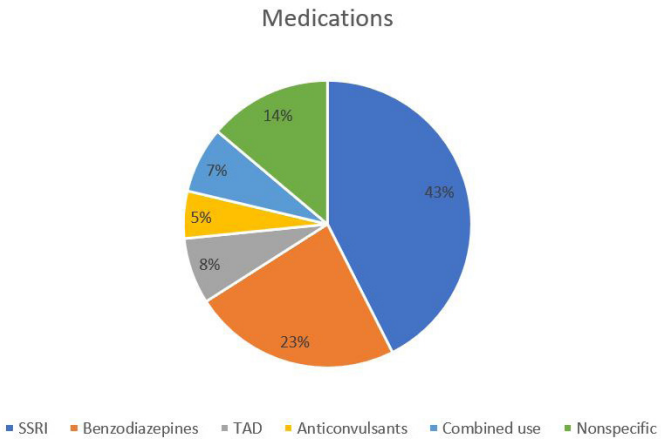
Vestibulometry

Of the total sample, 30 subjects (22.90%) presented dificulties in static balance and 94 (71.76%) dynamic balance. Among those without a psychiatric diagnosis (n = 57), 19 (14.50%) had dificulties in static balance and 17 (12.98%) had dificulties in dynamic balance. Among the subjects with psychiatric diagnosis (n = 74), 56 (75.68%) had dificulties in dynamic balance alteration and 11 (8.40%) had dificulties in static balance, and their relation was significant (Table 2).

Regarding the posturography evaluation, 128 subjects (97.71%) presented dificulties in a position, at least, with significant relation between the psychiatric diagnosis and lower values in the positions III and VI of the SOT (visual preference) and the relationship between the user group of psychotropics with lower values in positions III, V and VI of the SOT (Table 2).

It is possible to observe a prevalence of results within reference standards in the VENG tests in groups with a psychiatric condition, as well as those in a treatment with psychotropic medications, and no significant relationship was observed between these variables (Table 3).

In the caloric test, the result of normoreflexia prevailed (79.39%), that is, vestibular function within the reference standards, followed by labyrinthic predominance (7.63%), hyperreflexia (5.34%), hyporeflexia (4.58%) and directional predominance of nystagmus (3.05%).



Caption: SSRI - Selective Serotonin Reuptake Inhibitor; TAD - Tricyclic Antidepressants
Figure 2. Distribution of medications by type, used by subjects with psychiatric diagnosis (n = 51)

Table 1. Distribution and relationship between psychiatric diagnosis and psychotropic use and gender (n=131)

Gender	Psychiatric diagnosis		Psychotropic use	
	No	Yes	No	Yes
Women (n=109)	43 (39.45%)	66 (60.65%)	64 (58.72%)	45 (41.28%)
Men (n=22)	14 (63.64%)	8 (36.36%)	16 (72.73%)	6 (27.27%)
p	0.04		0.22	

Pearson's Chi-Square Test; $p \leq 0.05$

Table 2. Distribution of P-value, in the relationship between the group with dizziness only (Gt), dizziness and psychiatric diagnosis (Gt + d) and dizziness and psychotropic use (Gt + p) with alterations in static and dynamic balance tests and foam laser dynamic posturography (n = 131)

Evaluations		Results	Gt	Gt+d	*p*	Gt	Gt+p	*p*
Balance test	Static	+	38	63	0.01	58	43	0.12
		-	19	11		22	8	
	Dynamic	+	19	18	0.26	23	14	0.87
		-	38	56		57	37	
FLDP	SOT I	+	20	23	0.63	26	17	0.92
		-	37	51		54	34	
	SOT II	+	13	16	0.87	18	11	0.90
		-	44	58		62	40	
	SOT III	+	15	8	0.02	19	4	0.01*
		-	42	66		61	47	
	SOT IV	+	28	24	>0.05	34	18	0.41
		-	29	50		46	33	
	SOT V	+	25	22	0.09	34	13	<0.05
		-	32	52		46	38	
	SOT VI	+	21	13	0.01	27	7	0.01
		-	36	61		53	44	

Pearson's Chi-square test or Fisher's exact test*; $p \leq 0.05$
Caption: SOT - Sensory Organization Test; FLDP – foam laser dynamic posturography; (+) within the reference standards; (-)

Table 3. Distribution of p-value in the relation between the group with dizziness (Gt), psychiatric diagnosis (Gt+d) and psychotropic use (Gt+p) with alterations in VENG (n=131)

VENG		Gt	Gt+d	*p*	Gt	Gt+p	*p*
SN Open eyes	Absent	55	73	0.40*	78	50	0.66*
	Present	02	01		02	01	
SN Close eyes	Absent	48	62	0.95	68	42	0.69
	Present	09	12		12	09	
SSN	Absent	56	73	0.68*	79	50	0.63*
	Present	01	01		01	01	
Pendular tracking H	Type I/II	48	65	0.43*	68	45	0.28*
	Type III/IV	03	06		04	05	
Pendular tracking V	Type I/II	39	46	0.46	52	33	0.97
	Type III/IV	18	28		28	18	
Optokinetic nystagmus	Symmetric	49	69	0.17	70	48	0.17*
	Asymmetric	08	05		10	03	
RCT	Symmetric	53	67	0.43*	73	47	0.56*
	Asymmetric	04	07		07	04	
CalT	Normal	44	54	0.60	62	36	0.35
	Altered	11	17		15	13	

Pearson's Chi-square test or Fisher's exact test*; $p \leq 0.05$
Caption: SN - Spontaneous nystagmus; SSN - Semi-spontaneous nystagmus; H - Horizontal; V - Vertical; RCT- Rotatory Chair Test; CalT - Caloric Test

DISCUSSION

The high prevalence of pathological anxiety and diagnosis of depression was observed in this study, which corroborates with previous studies, showing a consistent combination of vestibular dificulties and a higher risk of psychiatric comorbidities, especially among women[13,15]. Dizziness, in the age group from 50 years (mean of the present study), can be influenced by the hormonal factors in the woman, that can provoke physiological, physical and/or emotional changes[16].

According to some authors, among patients with high scores in the Dizziness Handicap Inventory (DHI) questionnaire, there is a greater propensity for changes in the instrument that assesses the presence of anxiety and depression (HADS). Likewise, patients with anxiety and depression tend to have higher scores of DHI[17,18]. Moreover, psychiatric comorbidities are an important factor for the onset of dizziness[15]. These results, regardless of the triggering factor, reveal an intensifying action between anxiety/depression and dizziness.

The use of psychotropic medications in this sample was significantly higher than in the studies on the use of psychotropic medications in the general Brazilian population, 3.3% for benzodiazepine use[19], 5% or 5.2% for use without type specification[9]. These data indicate an important focus

for epidemiology in relation to the population of subjects with vestibular complaints.

There was a significant relationship between the use of psychotropic medications and a greater dificulty in the position V of the SOT, in which the main system evaluated was the vestibular. This relation raises some explanations, such as the subjects in use of psychotropics already present more symptoms, among them the dizziness, and, therefore, the drug treatment or effects of the medication itself.

In clinical practice, it is common for the patient with dizziness to report not following the prescribed treatment, that is, reduction of dose or suspension after symptom reduction. Regarding the side effects of antidepressant treatments, a presence of dizziness can be found among users who practice greater discontinuity of treatment[20].

In the present sample, the relationship between psychiatric diagnosis and dificulty in static balance tests was observed, but this relationship was not observed in the tests of dynamic balance. The presence of the complaint was also associated with positions III and VI of the SOT in Foam Laser Dynamic Posturography, in which vision is evaluated in a sensory overload condition. The majority of the sample did not present alterations in vestibulo-oculomotor function, observable by VENG, which reinforces data from the literature on non-vestibular causes in this population[5].

By not identifying alterations in reflexes and ocular motility in the oculomotor tests of the VENG, but in a behavioral evaluation (posturography), it inclines to explanations related to the visual processing of information, such as destabilization to visual overload. We could consider the possibility of being a side effect of psychotropic medications, however, of the 74 subjects with diagnosis, 24 were not taking medication and also presented lower values in positions III and VI, that is, in visual preference.

The organization of the vestibular system can be subdivided into three main functional groups: reflexive sensor-motor control of the gaze and balance - brainstem /cerebellum; self-perception and sensory-motor control of voluntary balance - cortical/subcortical level; and superior vestibular functions in which there is involvement of cognition or non-visual senses[21]. Some researchers suggest that the level of anxiety does not influence vestibular reflexes related to eye-head coordination. In addition, they claim that the perception of autorotation, based on vestibular information, is not modified by traits of anxiety. However, they indicate a different strategy for spatial orientation, suggesting that the use of vestibular information and its integration with representation of the body itself in space is affected by anxiety[22].

On the other hand, Halberstadt and Balaban (2006)[23] reported that the same neurons in the dorsal nucleus of rafe, which release serotonin, send projections to the amygdala as well as the vestibular nucleus of the brainstem. This result suggests that emotional changes can directly influence the processing of vestibular information.

In patients with dizziness, anxiety may possibly affect the interaction between visual, vestibular and somatosensory information during maintenance of postural balance, leading to greater anteroposterior axial instability[24]. This event is also observed among patients with panic syndrome, in whom increased sensory conflict may impede the functioning of the vestibular system leading to alterations in postural balance maintenance[25].

In previous research, with population similar to the present study, the data show that increased anxiety causes instability; such a condemnation is abolished with closed eyes. Therefore, anxiety can affect visual performance and influence sensory integration for postural control[26]. In addition, the vestibular system presents the connection with the cerebral cortex, hippocampus and amygdala regions. The loss of vestibular afferents can lead to impairments in these cognitive circuits, which are also related to emotion and affectivity[13].

The importance of investigating and monitoring psychiatric symptoms was explored in a study with patients with chronic dizziness, which reports a sustained reduction in vertigo symptoms in 78% of the sample through an otoneurological approach with coordinated vestibular rehabilitation to psychotherapeutics[27]. Moreover, depressed patients may feel more severely incapacitated by dizziness and instability than patients without depression[28].

Thus, it must understand depression or anxiety in subjects with dizziness, since these psychological states may exacerbate the symptoms. Beyond pharmacological therapy, behavioral therapy, such as vestibular rehabilitation, is an alternative that may be of great relevance in the treatment of this population[29].

The causal relationship between psychiatric conditions and the dizziness needs of future studies, especially longitudinal studies, that verify the influence of medication on body balance long-term, the mechanisms of dizziness, its relation of comorbidity, besides fomenting new scientific hypotheses about the etiologies and the development of new therapies.

This study has limitations. In the sample, there is a large difference between the number of women and men, which weakens an inferential analysis from the gender.

CONCLUSION

Based on this study, a high occurrence of the psychiatric condition among the subjects with dizziness was observed. The use of psychotropic medications in this sample of subjects with dizziness is substantially greater than general population, which reinforces the association between the psychiatric and vestibular complaints. The postural balance evaluation revealed a relationship between the anxiety/depression variables and a higher percentage of dificulties in Foam Laser Dynamic Posturography in the positions related to visual preference. However, no relationship was found between these conditions and alterations in the VENG tests.

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Author contributions

BNP was responsible of the study design, analysis of data and writing of the paper; VAVSF was the supervisor responsible for the study design and final revision of the manuscript.