Universidade do Minho
Escola de Ciências da Saúde

Dirceu Henrique Paulo Mabunda

Electroconvulsive therapy in psychotic patients: interface between perceived stress, anxiety and depression and the clinical outcome

Electroconvulsoterapia em pacientes psicóticos: interface entre o stress percebido, ansiedade e depressão e resultado clínico

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Prof. Doutor João Miguel Seiça Bessa Peixoto
e co-orientação do
Professor Doutor António Pacheco Palha

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«As for a future life, every man must judge for himself between conflicting vague probabilities.»

Charles Darwin

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Electroconvulsive therapy in psychotic patients: interface between perceived stress, anxiety and depression and the clinical outcome

ABSTRACT

Electroconvulsive therapy (ECT) continues to be considered an effective treatment of psychotic patients with hallucinatory and delusional symptoms resistant to antipsychotic medication. Several studies have evaluated the effectiveness of ECT in major depression as well as the effect of cortisol in the evolution and prognosis of those patients. However, studies that evaluate the evolution of the delusional and hallucinatory schizophrenic patients, psychotic depression and schizoaffective psychotic patients treated with ECT and its correlation with the salivary cortisol are scarce. Moreover, stress plays a significant role in modulation of mental disorders, and the hypothalamic-pituitary-adrenal (HPA) axis regulates the biological mechanisms of stress.

In this project we have assessed the clinical evolution of patients in three diagnostic groups, namely patients psychotic depression, schizoaffective disorder and schizophrenia before and after ECT. The Positive and Negative Syndrome Scale (PANSS) was used to evaluate psychotic symptoms, the Perceived Stress scale (PSS) to evaluate perceived stress, the Hospital Anxiety and Depression Scale (HADS) to evaluate anxiety and depression and salivary cortisol levels to evaluate the HPA axis function. The results of our study demonstrate that ECT is effective and a valuable therapeutic option which could be useful as adjunctive therapy in psychotic patients refractory to antipsychotics agents. There was a noticeable improvement in the PANSS, HADS and PSS scores after ECT in all psychotic patients in our study. Moreover, this study shows the effectiveness of ECT in chronic psychotic patients, while most studies have studied first psychotic episodes. The decrease of salivary cortisol after ECT in our study suggests that it may impact in the HPA axis, improving its function. However, the mechanism by which it can improve HPA axis function remains unclear. Finally, the correlations between variables revealed that the HADS score may predict the rate of response to ECT.

In conclusion, the present study has confirmed the role of ECT in the treatment of psychotic disorders. Importantly, a relation between HPA axis function and ECT was revealed specifically in patients with schizophrenia and psychotic depression but not in schizoaffective disorder, suggesting a distinct involvement of this key neurobiological factor.

Eletroconvulsoterapia em pacientes psicóticos: interface entre o stress percebido, ansiedade e depressão e resultado clínico

RESUMO

A eletroconvulsoterapia (ECT) continua a ser considerada um tratamento eficaz para pacientes psicóticos com sintomatologia delirante e alucinatória resistente à medicação antipsicótica. Vários estudos têm avaliado a eficácia da ECT na depressão major, bem como o efeito do cortisol na evolução e prognóstico desses pacientes. No entanto, estudos que avaliam a evolução em pacientes com esquizofrenia , depressão psicótica e psicose esquizoafetiva tratados com ECT e sua correlação com o cortisol salivar são escassos. Além disso, o stress desempenha um papel significativo na modulação de perturbações mentais sendo regulado pelo eixo hipotálamo-hipófise-adrenal (HPA).Neste projeto, avaliamos a evolução clínica dos pacientes em três grupos diagnósticos, nomeadamente pacientes com depressão psicótica, perturbação esquizoafetiva e esquizofrenia, antes e depois da ECT. A escala de sintomas positivos e negativos (PANSS) foi utilizada para avaliar sintomas psicóticos, a escala de stress percebido (PSS) para avaliar a percepção de stress, a escala de ansiedade e depressão hospitalar (HADS) para avaliar a ansiedade e a depressão e os níveis de cortisol salivar para avaliar a função do eixo HPA.

Os resultados do nosso estudo demonstram que a ECT é eficaz e uma valiosa opção terapêutica que pode ser útil como terapia adjuvante em pacientes psicóticos refratários a agentes antipsicóticos. Observou-se uma melhoria significativa nas pontuações PANSS, HADS e PSS após ECT em todos os grupos de pacientes psicóticos no nosso estudo. Além disso, este estudo demonstrou a eficácia da ECT em pacientes psicóticos crónicos, enquanto a maioria dos trabalhos têm estudado primeiros episódios psicóticos. A diminuição do cortisol salivar após ECT no nosso estudo sugere que esta tem um impacto sobre o eixo HPA, melhorando a sua função. No entanto, o mecanismo pelo qual ela modula a função do eixo HPA permanece desconhecido. Finalmente, as correlações entre as variáveis revelaram que os niveis de HADS podem predizer a taxa de resposta à ECT.

Em conclusão, o presente estudo confirmou o papel da ECT no tratamento de perturbações psicóticas. De realçar, a relação entre a função do eixo HPA e ECT foi revelada especificamente em pacientes com esquizofrenia e depressão psicótica, mas não na perturbação esquizoafetiva, sugerindo um envolvimento distinto deste factor neurobiológico.

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ABBREVIATIONS LIST

ECT- electroconvulsive therapy

HIV- human immunodeficiency virus

GABA- gamma amino butyric acid

NMDAR- N-methyl-D-Aspartate receptor

GluN1- Ionotropic glutamate receptors

GluA1- Glutamate receptor 1, family of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)

D1- dopamine 1 like receptor

GlyT1- glycine transporter

PFC- prefrontal cortex

COMT- catechol-o-methyl-transferase gene

5-HTTLPR- serotonin-transporter-linked polymorphic region

SERT- serotonin transporter

DAT- dopamine transporter

fMRI -functional magnetic resonance image

DTI- *Diffusion tensor imaging*

PET- Positron emission tomography

HPA-axis- hypothalamus-pituitary-adrenal axis

SSRI- Selective serotonin re-uptake inhibitors

D2- dopamine 2 receptor

5-HT1A - 5-hydroxytryptamine type receptor 1A

5-HT2- 5-hydroxytryptamine type receptor 2

BF- bifrontal electrode placement

BL- bilateral electrode placement

E-field- electrical field

IST- initial seizure threshold

BMI- body mass index

MAP- Medial Arterial Pressure

GRs- glucocorticoid receptors

MRs- mineralocorticoid receptors

PSS- perceived stress scale

HADS- Hospital Anxiety Depression Scale

PANSS- positive and negative syndrome scale

DSMV-TR- Diagnostic and Statistical Manual of Mental Disorders- text revised

UPY- unity pack year(number of cigarette smoked per day x number of years smoking/20)

RIA- radioimmunoassay

ELISA- enzyme linked immunosorbent assay

ECLIA- automated electrochemiluminescent assays

LCMSMS- liquid chromatographic methods coupled with mass spectrometry

IBL- international laboratory producer of cortisol kits

TMB solution- substrate solution, contains: TMB, buffer, stabilizers

TMB STOP- solution contain: 1M H_2SO_4(Sulphuric acid)

SPSS- Statistical Package for the Social Sciences

1. INTRODUCTION

1.1. Psychotic disorders

Psychotic disorders are psychiatric conditions characterized by specific psychopathological phenomena in the domains of thought and perception. In the domain of thought, changes can occur in thought content with the development of delusional ideas and in thought form manifested by disorganization of speech. In the perception domain, the expression of perceptions in the absence of external stimuli that are experienced in the external subjective space and cannot be controlled by the patient are defined as hallucinations which in association with delusions, constitute the backbone for he definition of psychosis. Furthermore, the lack of insight concerning the psychopathological phenomena described is important not only for diagnosis but also for treatment adherence.

In the diagnostic category of psychotic disorders, schizophrenia is the most common and well characterized disorder. Other psychotic disorders include paranoid disorder, substance-induced psychosis, schizoaffective disorder or shared psychotic disorder. Importantly, psychotic symptoms are often present in mania, depression and cognitive disorders (Thara, Taj, & Tirupati, 2008). Psychotic symptoms such as aggressive behavior, apathy and hallucinations have been recognized in Dementia, Parkinson's Disease and other degenerative disorders such as Huntington´s disease, motoneuron disease (Jellinger, 2012) and in infectious disorders such as HIV (Mohraz et al., 2014) and Syphilis (Allen et al., 2014).Patients with bipolar disorder may present high rates of psychotic symptoms associated with the core symptoms (Canuso, Bossie, Zhu, Youssef, & Dunner, 2008), which is associated with worse outcome of the disease (Mazzarini et al., 2010). Interestingly, coping strategies have been shown to contribute to better outcomes in patients with psychotics symptoms (Wigman et al., 2014). Finally, the duration of untreated psychosis is directly correlated with poor outcomes and with the severity of positive and negative symptoms (Tang et al., 2014), a fact that emphasizes the importance of treatment effectiveness and adherence.

1.1.1. SCHIZOPHRENIA: FROM THE ORIGIN TO NOWADAYS

Emil Kraeplin used the term 'dementia praecox' to describe the sub-acute development (Palha & Esteves, 1997) of a condition of mental weakness occuring at a youthful age. Kraeplin also distinguished dementia praecox from manic-depressive psychosis. In 1911, Bleuler renamed dementia praecox as schizophrenia, and thought that it resulted from splitting the psychic functions mostly cognitive and affective (Hunter & Woodruff, 2005). Bleuler described the four 'A's ' that were thought to be the core features of schizophrenia: autism, ambivalence, abnormal affect and loosening of associations (Tsoi, 2008).

Before the early descriptions by Kraeplin and Bleuler, negative symptoms were recognized as a one of principal features of the phenomenology of psychosis. Nowadays, negative symptoms such as blunted affect, apathy, alogy and anhedonia are recognized as a separate symptom domain and play important role as a mediator between cognition and functional outcome (Foussias, Agid, Fervaha, & Remington, 2013). Negative symptoms may be present in a wide range psychiatric disorders such as schizoaffective disorders, schizotypal personality disorder, major depression and have been linked with functional disability. Curiously, recent studies suggest that anhedonia may be considered as a different phenomena from negative and cognitive symptoms in schizophrenia(Huxley & Fonseca, 2014).

Schizophrenia is commonly diagnosed in young adulthood. At the time of first episode of psychosis, positive symptoms are generally improved by available antipsychotics, although negative symptoms and neurocognitive dysfunction seem to improve weakly with current psychopharmacological approaches. Recent studies suggests GABAergic-Glutamatergic balance via N-methyl-D-Aspartate receptor modulators, dopaminergic signaling and possibly, oxytocinergic and cannabinoidergic neurotransmission as potential targets to new pharmacological approaches (Millan, Fone, Steckler, & Horan, 2014). Furthermore, recent genomic studies suggests that inherited factors may be related with the abnormalities in white matter that are commonly described in schizophrenia (Duncan et al., 2014).

1.1.2. DOPAMINERGIC PATHWAYS:

Four distinct dopamine pathways act in synchronization in the brain: nigrostriatal, mesolimbic, mesocortical and tubero-infundibular (Reynolds, 2005). This complex dopaminergic system exerts different actions within several systems involved in motor function, motivation and reward attention, learning and memory ,and disruptions that can appear within this systems might lead to psychiatric disorders (Goto & Grace, 2007).In schizophrenic patients, studies have shown excessive dopamine in the striatum with concomitant depletion of dopamine in prefrontal cortex (Laruelle, 2014).

A recent study in anhedonic animals has shown that stress causes structural changes in mesolimbic pathways through neuronal morphological alterations in the nucleus accumbens, and that those changes are reversible after treatment with antidepressants (Bessa et al., 2013). Furthermore, recent data suggests that dopamine may play an important role in hippocampal neurogenesis via D1 like receptor (Takamura et al., 2014).

1.1.3. GLUTAMATERGIC PATHWAYS:

The serendipitous observation that the NMDA receptor (NMDAR) antagonists phencyclidine and ketamine, are able to induce psychotic symptoms opened a window to the hypothesis that glutamatergic signaling may provide an important therapeutical target in schizophrenia. Recent studies suggest that selective activation of metabotropic glutamate receptors mGlu5 and mGlu2 and muscarinic receptors, as well as blockade of glycine transporter GlyT1 may elicit antipsychotic effects in schizophrenia (Field, Walker, & Conn, 2011). Another study also suggests a role of D-serine in improved outcomes in schizophrenia patients (Labrie, Wong, & Roder, 2012). NMDAR´s are located in sub cortical regions on GABAergic neurons, and glutamatergic neurons in the PFC involved in the thalamocortical glutamatergic pathway (Vinson & Conn, 2012). NMDAR agonist drugs have shown to improve negative symptoms (Laruelle, 2014), positive symptoms, depressive symptoms and cognitive symptoms (Lin, Lane, & Tsai, 2012).

1.1.4. ETIOLOGY OF PSYCHOSIS

Recent studies have shown the role of genetic and epigenetic regulation in the etiology of psychosis namely in schizophrenia and bipolar disorder, with the identification of a common risk allele in chromosome 16p11.2 (Steinberg et al., 2014), Single Nucleotide Polymorphism (Bramon et al., 2014) and a role of low COMT hemizygosity (Gothelf et al., 2014). Furthermore, an increased risk of psychotic symptoms has been identified in patients with S-allele of 5-HTTLPR that predispose resistance to antidepressants (Stamm et al., 2013).Furthermore, post-mortem brain analysis in schizophrenia and depression subjects have shown alteration in histone methylation (Peter & Akbarian, 2011). Other studies have suggested a role of polymorphisms of SERT and DAT in resistance to treatment of psychosis (Bilic, Jukic, Vilibic, Savic, & Bozina, 2014). Genetic data analysis with bioinformatics tools and correlation with functional findings may advance our understanding of neurobiological mechanisms (Bray, Leweke, Kapur, & Meyer-Lindenberg, 2010).Finally, contradictory results have been published about the presence of neuronal surface antibodies in patients with primary psychiatric disorders (Coutinho, Harrison, & Vincent, 2014).

By using fMRI, DTI, cognitive data and biological markers researchers have demonstrate that it is possible to identify individual who have had a first episode psychosis, differentiating then this individuals with those with ultra high risk. This approach might be useful as diagnostic tool in clinical settings and health care services (Pettersson-Yeo et al., 2013).

Younger patients with psychotic depression and anxiety symptoms have shown improvement in psychotic symptoms when medicated with SSRI than older patients with the same clinical symptoms (S. J. C. Davies et al., 2014). Another study suggests that the deregulation of the HPA-axis in individuals with high risk of psychosis may be associated with depression and perceived stress in the psychotic episode (Thompson et al., 2007). Different early developmental pathways contribute for developing psychosis later in life in children with multiple developmental disorders (Sprong et al., 2008). Subjective reported negative attitude of others towards oneself was associated to vulnerability to psychosis (R K R Salokangas et al., 2012), and has been suggested has an early indicator of psychotic development (Raimo K R Salokangas et al., 2009).Another risk factor for psychotic depression is a familiar story of mental disorder, maternal

mental disorder increases risk off severe depression than a paternal disorder (Ostergaard, Waltoft, Mortensen, & Mors, 2013).

1.1.5. ANTIPSYCHOTICS

Classical antipsychotic drugs like Haloperidol are potent dopamine D2 receptors antagonists (Karam et al., 2010) while the more recent atypical antipsychotics like risperidone have less potentD2 receptor antagonism but also modulate different neurotransmitter receptors such as serotonin, noradrenalin and acetylcholine. Most of excitatory synapses in the central nervous system are formed onto dendritic spines (Glausier & Lewis, 2013).A neurochemical model for schizophrenia is the NMDA receptor hypo function hypothesis (Abi-Dargham & Laruelle, 2005), which proposes that NMDA receptor dysfunction may result in both positive and negative symptoms, as well as neurocognitive deficits (Davis, Horan, & Marder, 2013). Epigenetic modifications such as methylation or acetylation may also prove relevant (Ross, Margolis, Reading, Pletnikov, & Coyle, 2006). Studies have also suggested the use of drugs that modulate metabotropic glutamate receptors in the treatment of early phase of psychosis (Paz, Tardito, Atzori, & Tseng, 2008).

All antipsychotic medications are D2receptors antagonists, except aripiprazole (Brosda, Jantschak, & Pertz, 2014) which exhibits partial agonist effect at D2 receptor and demonstrated effectiveness for both positive and negative symptoms, improving cognitive and memory via action on 5-HT1A receptors (S. Leucht et al., 2012). Atypical antipsychotics exhibit efficacy in treatment of positive symptoms with low extrapiramidal side effects, due to its core features of 5-HT2 antagonism and D2 antagonism (Hood, Orr, & Nutt, 2007). Quetiapine, iloperidone and malperone, increase dopamine and acetylcholine release in pre frontal cortex through partial agonism on 5-HT1A receptor (Ichikawa, Li, Dai, & Meltzer, 2002). Recent studies suggests important role of clozapine (Gemperle, Enz, Pozza, Lthi, & Olpe, 2003) in long-term potentiation and demonstrate an correlation between volume of basal ganglia and treatment with risperidone (Hutcheson, Clark, Bolding, White, & Lahti, 2014).

Studies suggests that haloperidol affects neuroplasticity playing an important role in synapse formation and rearrangement (Konradi & Heckers, 2001). Recent data confirms that

antipsychotics differ from each other in terms of efficacy (Samara, Cao, Helfer, Davis, & Leucht, 2014), while other study suggests that depot antipsychotic are clinically superior compared to oral in outpatients by reducing relapses rates (C. Leucht et al., 2011). Despite recent advances in neuroscience and psychopharmacology, antipsychotic treatment does not improve outcome in about 30 % of psychotic patients (Nguimfack, 2004). In these refractory cases ,electroconvulsive therapy (ECT)remains the last and most effective therapeutical approach (Fablet-Vergnaux, Loirat, & Vanelle, 2003).

1.2. Electroconvulsive Therapy:

Electroconvulsive therapy (ECT) is a non-pharmacological biological treatment consisting of the successive application of an electrical current in the human brain, for the treatment of a mental disorder (Thirthalli, Prasad, & Gangadhar, 2012).

Giovanni Aldini was the first to use electricity to treat melancholia in 1801, and at that time the electricity was used as a punishment method. In 1937, Professor Lucio Bini due to serendipity reported the use of electricity to safely induce seizures in dogs. Hugo Cerletti, chairman of Bini, developed the task to improve the use of electric stimulation for human approaches. That discovery constituted a therapeutic revolution, and in august of 1939, at the 3rd International Neurological Congress in Copenhagen, Professor Bini reported the first use of electrical stimulation in psychotic patients for therapeutical purposes (Faedda et al., 2010). The recognition of the therapeutic effect of complete seizures belongs to von Meduna, although the concept of induce seizure through chemical means had been used since the 16th century (Payne & Prudic, 2011).

1.2.1.MECHANISM OF ACTION OF ECT:

The mechanism of action of ECT remains unknown, despite the major advances in the field of neuroscience. Studies have shown a reduction of 5-HT_{1A} receptor binding in hippocampus,

amygdala, cingulate and orbitofrontal cortices of depressed patients after ECT (Lanzenberger et al., 2013) and effectiveness in delusional depression, psychosis and catatonic conditions, suggesting that ECT shares similar molecular mechanisms with antidepressant treatments (Gersner, Toth, Isserles, & Zangen, 2010). An anorexigenic effect of ECT mediated by the ventromedial hypothalamus has also been demonstrated (Segi-Nishida et al., 2013). ECT also stimulates proliferation of endothelial cells in the hypothalamus, improving hypothalamic dysfunction associated with mood disorder and psychosis (Jansson, Hellsten, & Tingström, 2006).A recent study reveals important effects of ECT in the limbic system, demonstrating an increase of amygdala and hippocampus volumes after ECT (Tendolkar et al., 2013).

Recent studies demonstrated that ECT generates different pattern of electrical field in portions of the brain. Bifrontal ECT(BF) generates higher E-field in prefrontal structures compared to bilateral and right unilateral ECT. On the other hand, BL ECT produces higher E-field in thalamus and hypothalamus this pattern exert an role in superior antidepressant efficacy of BL ECT (Lee et al., 2012). The initial seizure threshold (IST) should be defined based on all parameters, not only in the summary metric (Peterchev & Lisanby, 2010). IST of 68,2 mC is considered within the stimulus dose range of 'fixed dose' method (van Waarde, Verwey, & van der Mast, 2009). Clinical symptoms were resolved with ECT in an PET study of cerebral glucose consumption with shifts in the balance of corticolimbic function (Suwa et al., 2012).

1.2.2.ECT TECHNIQUE:

In ECT practice three electrode placement are commonly used: bitemporal, bifrontal and right unilateral configurations. A recent review shows that bilateral electrode placement is the more efficient placement method (Fink, 2014).

Neurons depolarize in a width in the order of 0,1 to 0,2 milliseconds (ms).Pulse width lower than 0,5 milliseconds is considered ultra brief-pulse ECT, pulse width 0,5-2,0 milliseconds range are considered brief pulse ECT (Bai, Loo, Al Abed, & Dokos, 2012).,Excessive pulse width should be avoided, as this energy administered during refractory period may cause cognitive impairments (Sackeim, Prudic, & Lisanby, 2008). Studies have shown no significant differences in efficacy between ECT administered twice or three tome per week (Siskind, Charlson, Saraf, Scheurer, & Lie, 2012)(Siskind et al., 2012).

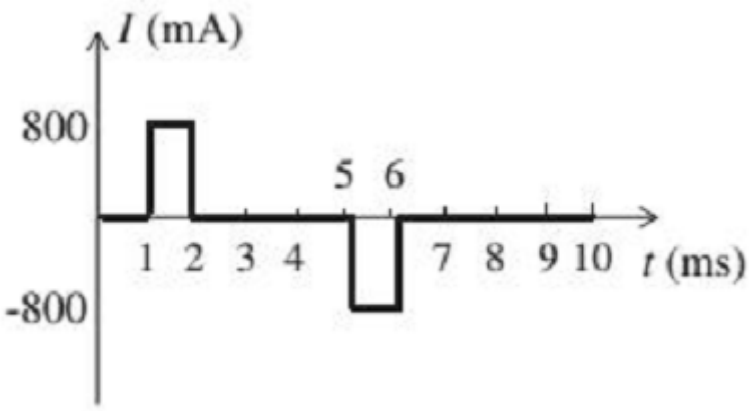


Figure 1.Schematic representation of biphasic brief pulse Adapted from (Bai et al., 2012).

1.2.3.ECT EFFECTIVENESS:

Schizophrenic patients show higher short-term improvement in symptomatic and cognitive outcomes in BF ECT than in BT ECT (Phutane et al., 2012).A case-control study demonstrated security and safety of ECT in first-episode psychosis, with quickly clinical improvement and shorter hospitalization (Zhang et al., 2012). Nevertheless, a controversy remains about the impact of ECT in neuroendocrine and immunological patterns. While some studies show that acute ECT does not alter neuroendocrine-immune function (Fernandes et al., 2009)(Fluitman et al., 2011), others show an alteration in neuroendocrine function (Haghighi et al., 2013)(Stelzhammer et al., 2013).A recent study revealed that patients with higher body mass index increase more the systolic blood pressure (SBP) after ECT, and this elevation in SBP after ECT was linearly related to BMI (Takagi, Iwata, & Nakagawa, 2012). Bilateral ECT in schizophrenia results in hemodynamic changes in PFC with left dominant asymmetric alteration (Fujita et al., 2011). Finally, ECT can be considered a secure and safe adjunctive treatment according to a recent study that shows an efficacy in preventing recurrence of depression episodes without cognitive impairment over several years (Elias, Chathanchirayil, Bhat, & Prudic, 2014).

1.2.4. INDICATIONS OF ELECTROCONVULSIVE THERAPY

ECT is effective in the treatment of different psychiatric disorders, including major depression, mania, schizophrenia and schizoaffective disorder (Baghai & Moller, 2008). It is also indicated in the treatment of psychiatric disorders in pregnancy (Bulbul et al., 2013) by presenting a minimal

risk to the fetus and pregnant women (Anderson & Reti, 2009). Schizophrenic patients who do not respond to pharmacological treatment (Lévy-Rueff, Gourevitch, Lôo, Olié, & Amado, 2010) show improvement of psychotic symptomatology with ECT (Shimizu et al., 2007)(Zervas, Theleritis, & Soldatos, 2012). Recent studies have shown that ECT is more effective than repetitive transcranial magnetic stimulation in the treatment of maintenance in schizophrenics with hallucinatory activity (Matheson, Green, Loo, & Carr, 2010), resistant psychotic depression and schizoaffective disorder (Ren et al., 2014).

1.3. Stress and psychosis:

1.3.1. THE ROLE OF HPA-AXIS

In acute stressful conditions, adaptative mechanisms mediated by the HPA-axis and facilitation of the ventral route to the amygdala enhances emotional arousal. On the other hand, chronic stress has negative effects by reducing the surface expression of glutamate receptors in prefrontal cortex (GluN1 , GluA1), leading to a disruption of excitatory synaptic transmission and neuroplasticity. Genetic background which can serve as a resilience factor or vulnerability factor plays an important role in psychopathology development (Timmermans, Xiong, Hoogenraad, & Krugers, 2013). Stress can be present before the first psychotic episode and confer vulnerability to the patient(van Venrooij et al., 2012) and can be a trigger of psychotic symptoms (Tso, Grove, & Taylor, 2012).

Salivary cortisol is one of the most reliable indicators of the levels of activation of the hypothalamic-pituitary-adrenal and is better to shown the neuroendocrine effects of ECT as its correlation with plasma cortisol is about 0,7 (Obayashi, 2013) and does not suffer rapid variations (Corcoran et al., 2003). Previous studies have shown that in patients with schizophrenia, negative symptoms were not associated with low levels of self-reported stress (A. S. Cohen, Docherty, Nienow, & Dinzeo, 2003) and that the acute reaction stress is delayed or reduced in these patients (K Brenner et al., 2009).

The deregulation of the hypothalamic-pituitary-adrenal axis (HPA) plays an important role in the psychosis onset and course of schizophrenia (van Winkel, Stefanis, & Myin-Germeys, 2008). Cortisol is a corticosteroid hormone that acts in stress response and neuronal networks (Corcoran et al., 2003). The bind of cortisol to glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) can induce changes in neuronal function. Mineralocorticoids receptors have higher affinity for cortisol than glucocorticoids receptors. The hippocampus contains MRs and GRs, these receptors also are expressed in cortical layers and various brain regions. The persistent elevated levels of cortisol can be neurotoxic, as previous studies have shown reduction of hippocampal volume (Walker, Mittal, & Tessner, 2008), and cognitive impairments with memory deficits. A recent study has shown low biological stress response in medicated patients, indicating that impairments in stress mechanisms may be considered an endophenotype or vulnerability factor in schizophrenia (van Venrooij et al., 2012). Stress activated complex neuronal circuitry, and the current research are centered on genetics factors that may explain differences in stress response (Sousa & Almeida, 2012).

A study in patients with major depression showed that the improvement of the clinical picture after ECT was higher in patients who had higher levels of cortisol after dexamethasone suppression (Vukadin, Birkenhäger, Wierdsma, Groenland, & van den Broek, 2011). Studies have shown that the levels of the neurosteroid dehidroenpiandrosterone sulphate, are predictors of response to ECT in psychotic patients (Maayan et al., 2000). However, the relationship between cortisol levels and the therapeutic response to ECT has not yet been studied in schizophrenia (Kronfol, Hamdan-Allen, Goel, & Hill, 1991). Neuroleptic treatment, in particular atypical antipsychotics, has shown to be effective in restoring some of the cognitive dysfunction and negative symptoms in psychotics patients. However, the effects of ECT is not well studied in these symptoms (van Venrooij et al., 2012).

1.4. Final considerations

Electroconvulsive therapy is still the most effective treatment for patients psychotic disorders that are resistant to the various psychopharmacological approaches. The mechanism of ECT action remains unknown, despite the advances and new approaches reached in the last decades with animal models and neuroimages studies. Thus, understanding how ECT acts to relief and

improve psychotics symptoms such as delusions and hallucinations might be essential to improve current psychopharmacological tools, and develop new drug target.

2. OBJECTIVES

General: Using an observational study in clinical settings, we were able to better understand the relations between stress, anxiety and depression amongst patients with psychotic symptoms with indication to perform ECT.

Specifics:

The present work aim to:

1- Evaluate the evolution of positive and negative symptoms in psychotic patients undergoing ECT.
2- Assess clinically perceived stress, anxiety and depression in these patients, with the use of PSS scale and HADS scale.
3- Correlate salivary cortisol levels with disease activity and progression, evaluated in terms of clinical evolution after ECT.

3. MATERIAL AND METHODS

3.1. SAMPLE:

All procedures were conducted according to the ethical principles, with local hospital authorizations and patients included in study assigned informed consent. A total of 36 inpatients were included in the study, from Casa de Saúde do Bom Jesus in Braga and from Hospital de Magalhães Lemos in Porto from October 2013 to April 2014. All participants had formal indication to perform ECT, and were divided according to three psychiatric diagnosis (DSMIV-TR): Psychotic depression, Schizoaffective Disorder and Schizophrenia.

3.1.1. INCLUSION CRITERIA:

-Diagnosis of Schizophrenia, Psychotic depression or Schizoaffective disorder according to DSMV-TR.

-Clinical Criteria to carry out ECT

-Age between 20 and above.

-Sign informed consent

3.1.2. EXCLUSION CRITERIA:

- Infection at the time of study,

- Substance abuse or dependence(alcohol, cannabis)

- Organic impairment, such as : Dementia,

- significant cardiovascular, respiratory conditions.

-Epilepsy or other neurological conditions,

- Brain tumors or lesions space-occuping intracranial.

3.2. TASKS:

Task 1 – Evaluation of the effect of ECT in the evolution of delusional symptoms

The presence, intensity, frequency, quality and characteristics of the hallucinatory and delusional symptoms of patients who accepted to participate in the study and fulfill the inclusion criteria were assessed retrospectively using the clinical process prior to treatment, and compared with similar data collected prospectively through a weekly evaluation on four weeks after the end of treatment. In addition the Positive and Negative Syndrome Scale (PANSS)were also applied to each patient, before the treatment and after the end of the treatment, comparing the results in both times to each individual (Rami et al., 2008). Social demographic data were also collected, which were related with PANSS scores for each patient.

Task 2 -Cortisol in patients undergoing ECT, relation with stress, anxiety and depression.

To evaluate the state of stress exposure salivary cortisolwas measured (Karene Brenner, St-Hilaire, Liu, Laplante, & King, 2011). The samples were collected with Salivette devices in fasting (at 09:00 a.m) in the week preceding the implementation of ECT and in the week after the end of treatment. Samples were subsequently centrifuged and frozen at -86ºC until the time of analysis with a commercial ELISA kit for cortisol. Cortisol levels were analyzed and correlated with the PSS score and HADS score applied in the collection time.

Task 3- ECT techniques

ECT procedures were conducted by a multidisciplinary team composed of an anesthesiologist, psychiatrist and nurse. Bitemporal electrode placement ECT was performed in all patients for being effective and with fewer side effects (Phutane et al., 2012). The number of ECT treatment sessions ranged from 6 -10, twice or three time per week, using a modified protocol with propofol for anesthetic and suxamethonium to induce muscle relaxation.

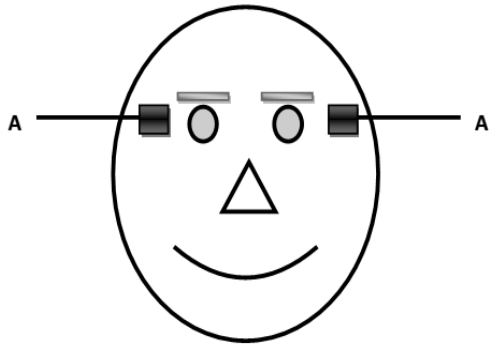


Figure 2 Schematic representation of electrode placement.(A)- position of electrodes for bitemporal ECT. Electrodes are positioned symmetrically on either side of the forehead just 3cm above midpoint of line running from the outer cantus of the eye to the external auditory meatus.

3.3. EXPERIMENTAL DESIGN

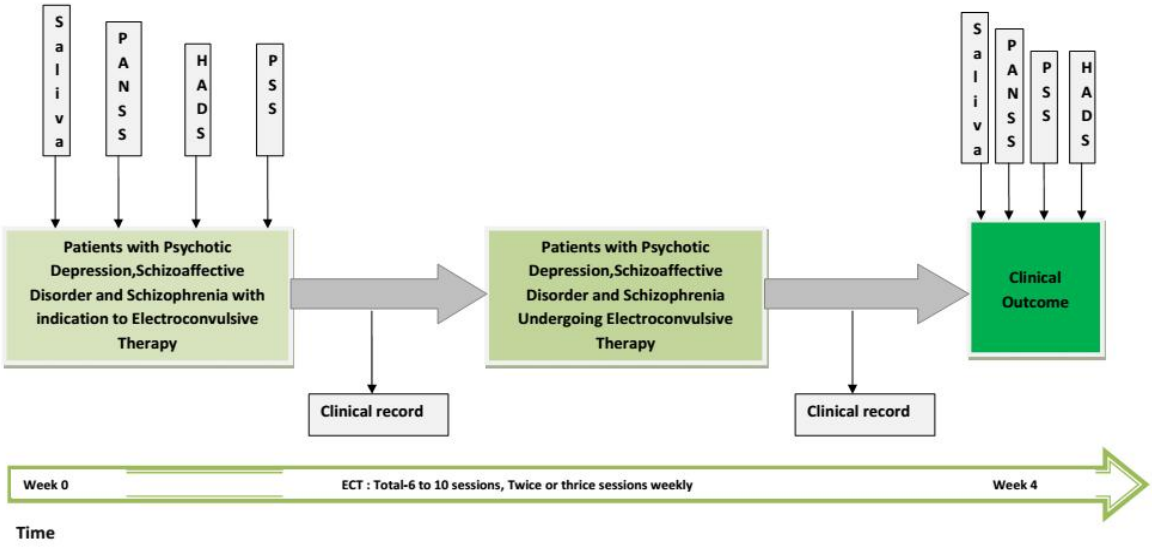


Figure 3Schematic representation of experimental design

3.4. VARIABLES ASSESSMENT

Alcoholic habits were measured by quantity of alcohol intake per day, and converted to unit g/l. Smoke habits, measured by unity pack year (UPY), were calculated with the formula: UPY=number of cigarette smoked per day x number of years smoking/20. The weight and height

were transformed to Body Mass Index by the following formula: BMI=weight/(height)^2 , weight(kilograms) and height (meters). The BMI is the most accurate (Melmer et al., 2012) to predict anthropometric parameters, glucose, lipidic homeostase and blood pressure . The cardiac rate, systolic and diastolic blood pressure were assessed. Patients with schizophrenia have shown low cortisol reaction when submitted to psychological stressors, but the heart rate and blood pressure increases in these patients(K Brenner et al., 2009).

3.4.1. CLINICAL VARIABLES:

The clinical file of the patients was used to collect the following variables: years passed since the first diagnoses of psychosis and the last outbreak of disease. Psychiatric diagnosis according to Diagnostic and Statistical Manual of Mental Disorders-Text Revised (DSMIV-TR). Symptoms before the ECT and symptoms after ECT. Psychological follow up, psychiatric medication in course.

3.5. CORTISOL SAMPLING:

Patients were trained (not to eat and do not ingest liquid 30 minutes prior to harvest) to take samples of saliva (Restituto et al., 2008) at 09:00 o'clock in the morning, and each one received a Salivette® (Sarstedt, Numbrecht, Germany). The samples were centrifuged at 2000rpm for 2 minutes, and stored at -86 °C in aliquots until analysis. Salivary cortisol follows the diurnal plasma and serum rhythm cortisol and reflect biological free-cortisol. To collect the sample, an absorbent swab was applied in the mouth for 30 seconds to3 minutes until saturation and then transferred into the tube. Several assay techniques(Inder, Dimeski, & Russell, 2012) to measure salivary cortisol are available: radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), automated electrochemiluminescent assays (ECLIA) and liquid chromatographic methods coupled with mass spectrometry (LCMSMS). The advantage of ELISA is that it is a more convenient method, with low cost, specificity and does not require expertise. The disadvantages are that it is less sensitive than LCMSMS and the possible cross-reactivity with other steroids.

3.5.1. PRINCIPLE OF ASSAY

In competitive assay labeled antigen binds to antibody-binding sites unoccupied by sample antigen. The addiction of sample antigen in the system leads to a reduction in the number of the

free binding sites(C. Davies, 2013). Three factors contribute to sensitivity of the assay: the equilibrium constant, the signal measurement and the level of non-specific binding.

3.5.2. TEST PROTOCOL

Cortisol ELISA kits (RE52611) produced by IBL-international GMBH were used. The reportable range for salivar cortisol is 0,015 - 4 μg/dl cortisol.

Table 1. Procedure of ELISA

STEP	Procedure
1	The tubes with sample, control and standard were vortexed and added 50μl of each into the respective wells of the plate.
2	Vortexed and added 50μl of enzyme conjugate into each well, cover plate with adhesive foil, shaked plaque carefully.
3	Incubated for 2 hours at room temperature, on a orbital shaker (400-600 rpm).
4	Were removed adhesive foil. Discarded incubation solution. Washed the plate 4 times with 250μl of diluted wash buffer. Removed excess of solution in each time by tapping the inverted plate on a paper towel.
5	Added 100μl of TMB substrate solution into each well.
6	Incubated plate, for 30minutes, at room temperature on an orbital shaker(400-600 rpm)
7	Added 100μl of TMB STOP solution into each well to stop the substrate reaction. Color changed from blue to yellow. Shaked briefly
8	Optical density were read in a photometer at 450 nm (reference wave length- 600-650) within 15 minutes after added STOP solution

3.6. THE POSITIVE AND NEGATIVE SYNDROME SCALE ASSESSMENT(PANSS)

PANSS was assessed in two time points, before the first ECT , and one week after the last ECT, to measure the positive and negative symptoms. PANSS is a good instrument to assess both negative and positive symptoms (S. Leucht et al., 2005) and general psychopathology. It includes psychometric items with higher reliability (Peralta & Cuesta, 1994), validity and evaluates the pharmacological effect of treatment (Kay, Fiszbein, & Opler, 1987)sensitivity. A recent study (Santor, Ascher-Svanum, Lindenmayer, & Obenchain, 2007) has shown that the positive and negative subscales are more sensitive to change, the PANSS items are good to assess severity of disease.

3.7. PERCEIVED STRESS SCALE ASSESSMENT

PSS was assessed before the first ECT, and one week after the last ECT. The scores were correlated with the salivary cortisol levels, heart rate, mean blood pressure and symptoms. The PSS is an objective measure of self report and important tool to assess the stress levels in patients (S. Cohen et al,, 1983). The main advantage is that it allows to measure the risk of disease, with events that occurred during the last month and minimize bias of subjective measures (S. Cohen et al., 1983). A study has shown that patients with negative symptoms of schizophrenia have low capacity to experience stress (A. S. Cohen et al., 2003), and suggests that stress may not cause relapses of negative symptoms. Other study showed that patients with high PSS score have lower levels of cortisol after awaking than patients with low PSS score(Maldonado et al., 2008).

3.8. HOSPITAL ANXIETY AND DEPRESSION SCALE ASSESSMENT

The Portuguese version (Pais-Ribeiro et al., 2007) of the Hospital anxiety and Depression Scale (HADS).was administered to each patient who agreed to participate in the study. HADS self-assessment with fourteen items measures symptoms of anxiety and depression of the past 7 days, with the score of each item ranging from 0 to 3. Scores above 8 indicate severe depression or anxiety. This scale is important for research purposes(Herrmann, 1997) as it characterizes the prevalence and intensity of depression and anxiety. HADS is limited to 14 items which makes it easy to use. We used 8 for both anxiety and depression as a cutoff point.

3.9. STATISTICAL ANALYSIS

All statistical analysis was conducted in the SPSS software package version 22(IBM corporation, New York). Normality of data sets was assessed using the Kolmogorov-Smirnov test, Kurtosis and Skweness. As our data met normality, parametric tests were used throughout the analysis. Comparisons means between and within groups of diagnosis(Psychotic Depression, Schizophrenia and Schizoaffective Disorder) were performed using the ANOVA REPEATED MEASURE analysis. Pearson correlations were computed between continuous variables. The demographic, clinical, psychometric and laboratory were reported with use of descriptive statistics (frequencies, averages and standard deviation).

4. RESULTS

4.1. CLINICAL DIAGNOSTIC AND SOCIO-DEMOGRAPHIC CHARACTERISTICS:

The clinical and socio-demographic profile of the subjects was assessed with the use of descriptive statistics. During the study period, 36 patients were included, in which 17 patients were diagnosed with Schizophrenia (47,2%), 10 with Psychotic Depression (27,8%) and 9 with Schizoaffective Psychosis (25%). The diagnosis was established according to the Diagnostic and Statistical Manual for Mental Disorders-TR (DSMIV-TR) by a senior psychiatrist. All patients were physically healthy with no infection or history of drug or alcohol abuse. The mean age of the patients was 49,22 years (sd=15,130). The duration of the psychiatric disorders was 14,67 years (sd=12,191). The mean education level of our patients was 7,64 years (sd=3,972).

Twenty three patients were female (63,9%) and thirteen were male (36,1%) and all of them were medicated with antipsychotics. Our sample displayed a mean body mass index (BMI) of 26,5710 kg/m^2 (sd=4,00862) and mean arterial pressure (MAP) of 92,5648mmHg (sd=10,93264) before ECT. No significant differences were observed in these measures after ECT.

4.1.1. RESPONSE TO ECT AMONG PSYCHOTIC PATIENTS

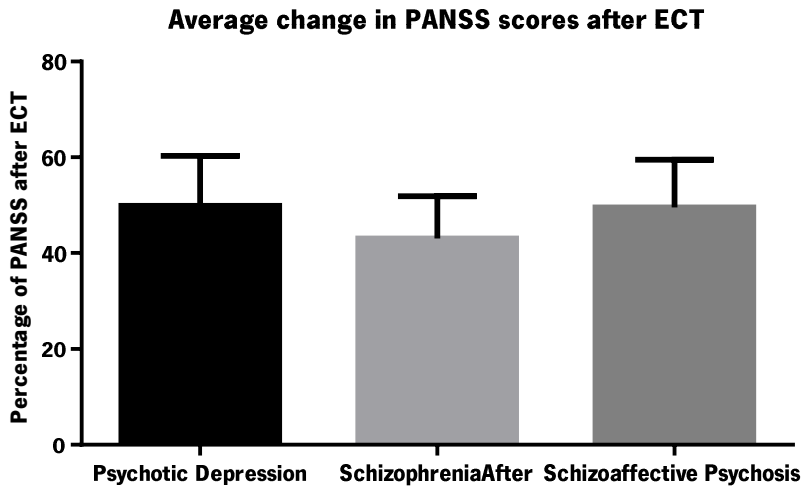


Figure 4. Average change in PANSS scores after ECT in the different experimental groups

The analysis of the clinical response to ECT revealed a decrease in the PANSS scores in the three experimental groups (Figure 4). However, no significant differences were observed between psychotic patients in the average change in PANSS scores, demonstrating the efficacy of this treatment independently of the diagnosis.

Table 2. Effectiveness of ECT on PANSS

Descriptive statistics of differences of PANSS between psychotics patients undergoing ECT

Psychotic Disorder		Mean	Std. Deviation	N
PANSSbefore	Schizophrenia	127,35	16,733	17
	Schizoafective_p	124,56	17,016	9
	Psychotic_Depres	104,30	9,334	10
	Total	120,25	17,848	36
PANSSafter	Schizophrenia	55,35	16,175	17
	Schizoafective_p	61,44	13,408	9
	Psychotic_Depres	52,10	11,666	10
	Total	55,97	14,417	36

The descriptive statistics of the differences in PANSS scores (table 2) reveal that the mean score of PANSS was lower after ECT in all three psychotic patients' groups. To further analyze the differences between the three psychotic patients' groups, Anova Repeated Measures was performed with Tukey post-hoc test. Our data reveale statistically signifcant differences between three groups in PANSS scores, HADS scores and Perceived Stress.

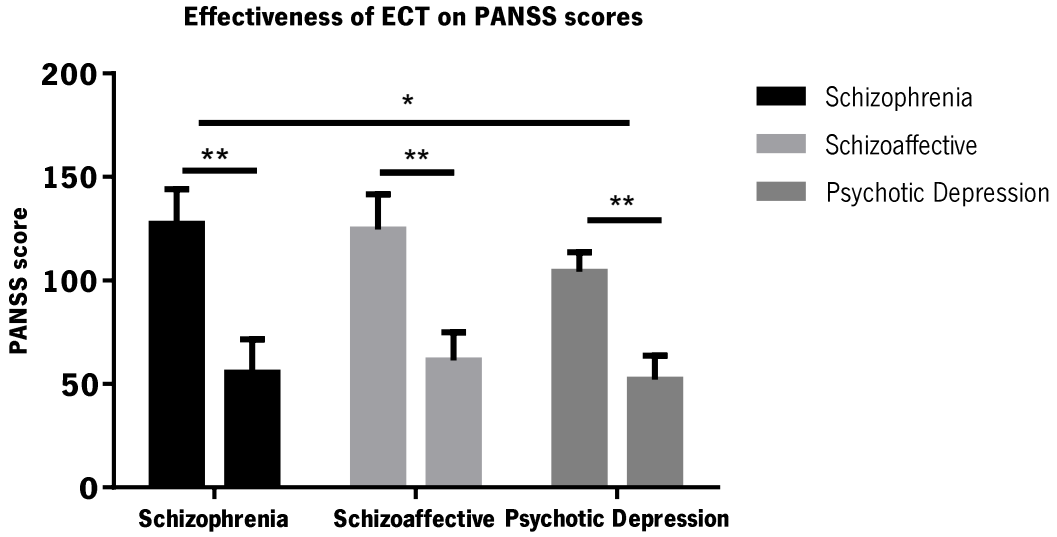


Figure 5. Effects of ECT on PANSS score *p value less than 0,05. **p value less than 0,01.

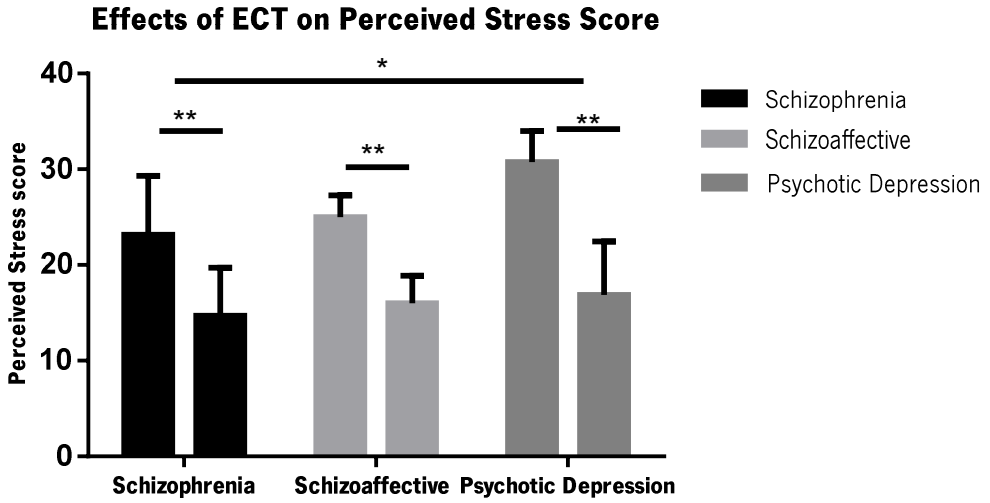
The repeated measures ANOVA analysis revealed a significant global effect of ECT on the PANSS scores F(2,33)=6,948, p=0,003. The Tukey post-hoc test revealed a significant difference in PANSS scores between schizophrenic patients and psychotic depression one week after ECT (p=0,045) but no significant differences between schizophrenic patients and schizoaffective patients (p=0,951) and psychotic depression and schizoaffective patients (p=0,051)(Figure 5).

4.1.2. EFFECTS OF ECT ON PERCEIVED STRESS

Table 3. Descriptive statistic of perceived stress

Psychotic disorder		Mean	Std. Deviation	N
PSS_befor	Schizophrenia	23,18	6,126	17
	Schizoafective_p	25,00	2,291	9
	Psychotic_Depres	30,80	3,225	10
	Total	25,75	5,628	36
PSS_after	Schizophrenia	14,71	5,022	17
	Schizoafective_p	16,00	2,872	9
	Psychotic_Depres	16,90	5,567	10
	Total	15,64	4,722	36

The descriptive statistics of the differences in PSS scores (table 3) reveal that the mean score of PSS was lower after ECT in all three psychotic patients' groups.



6.Effect of ECT on perceived stress. *p value less than 0,05. **p value less than 0,01.

The repeated measures ANOVA analysis revealed a significant global effect of ECT on the PSS scores F(2,33)=4,841 p=0,014. The Tukey post-hoc test revealed a significant difference in PSS scores between schizophrenic patients and psychotic depression one week after ECT (p=0,015) but no significant differences between schizophrenic patients and schizoaffective patients (p=0,639) and psychotic depression and schizoaffective patients (p=0,202)(Figure 6).

4.1.3. EFFECTS OF ECT ON ANXIETY AND DEPRESSION SCORES

Table 4. Descriptive statistic of HADS

Psychotic Disorder		Mean	Std. Deviation	N
HADS_before	Schizophrenia	21,41	8,675	17
	Schizoafective_p	25,56	6,560	9
	Psychotic_Depres	33,30	5,034	10
	Total	25,75	8,729	36
HADS_after	Schizophrenia	11,53	5,076	17
	Schizoafective_p	12,11	6,234	9
	Psychotic_Depres	14,50	4,327	10
	Total	12,50	5,207	36

The descriptive statistics of the differences in HADS scores (table 4) reveal that the mean score of HADS was lower after ECT in all three psychotic patients' groups.

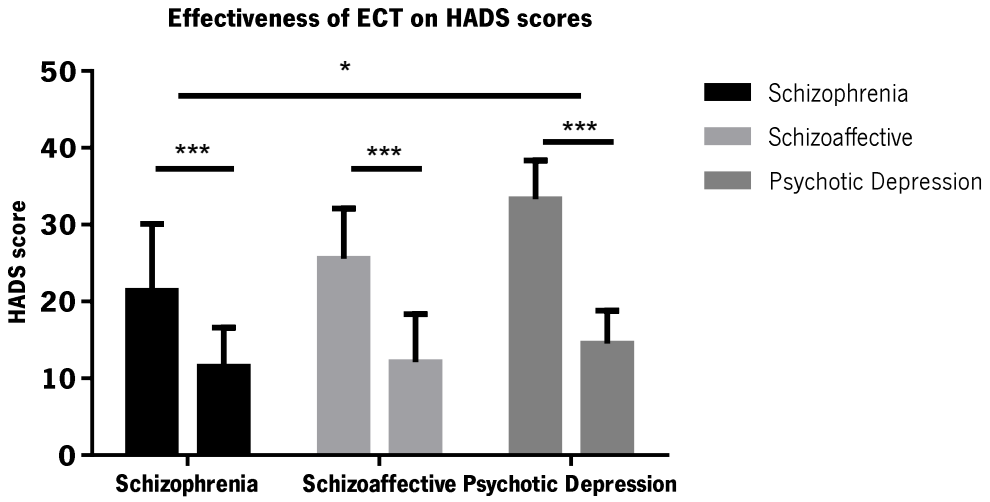


Figure 7. Effectiviness of ECT in Anxiety and Depression. *p value less than 0,05. **p value less than 0,01.

The repeated measures ANOVA analysis revealed a significant global effect of ECT on the HADS F(2,33)=6,575 p=0,004. The Tukey post-hoc test revealed a significant difference in HADS scores between schizophrenic patients and psychotic depression one week after ECT (p=0,006) but no significant differences between schizophrenic patients and schizoaffective patients (p=0,563) and psychotic depression and schizoaffective patients (p=0,132) (Figure 7).

4.1.4. EFFECTS OF ECT IN SALIVARY CORTISOL

Table 5.Descriptive statistic of salivary cortisol

DSMIV_tr		Mean	Std. Deviation	N
Cortisol_Before	Schizophrenia	,81050	,411265	16
	Schizoafective_p	,73025	,331653	8
	Psychotic_Depres	1,05780	,763147	10
	Total	,86435	,525503	34
Cortisol_After	Schizophrenia	,57069	,232323	16
	Schizoafective_p	,62525	,203408	8
	Psychotic_Depres	,56820	,380697	10
	Total	,58279	,270941	34

The descriptive statistics of the differences in salivary cortisol levels (table 5) reveal that the mean levels of cortisol after were lower in the schizophrenia and psychotic depression groups.

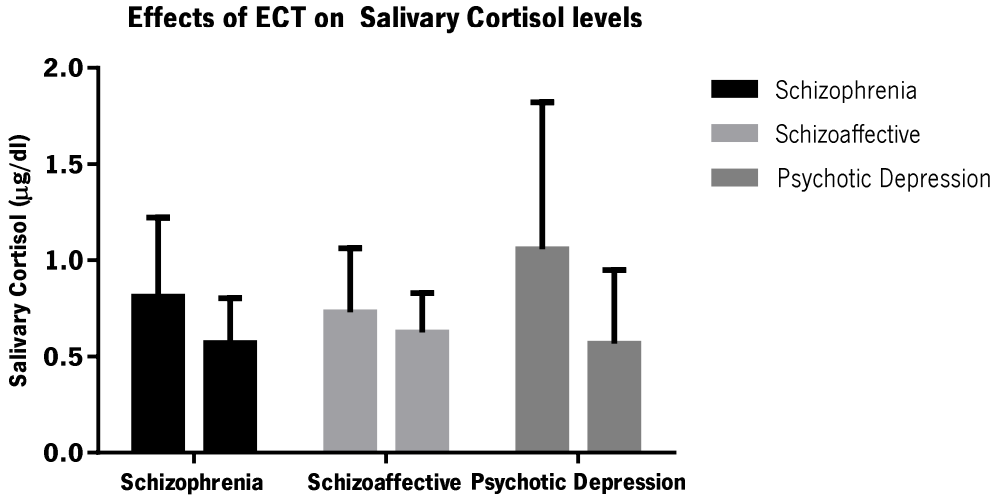


Figure 8. Effects of ECT on salivary cortisol levels among psychotic patients

The repeated measures ANOVA analysis revealed that there were no significant global effects of ECT on the salivary cortisol levels F(2,31)=2,180 p=0,130 (Figure 8).

4.2. DIFFERENCES IN CLINICAL OUTCOME BETWEEN GENDER

Table 6. Descriptive statistic of differences between gender

gender		N	Mean	Std. Deviation	Std. Error Mean
age	Male	13	41,23	16,300	4,521
	Female	23	53,74	12,657	2,639
PANSSafter	Male	13	49,31	9,160	2,540
	Female	23	59,74	15,615	3,256
PSS_befor	Male	13	23,46	7,102	1,970
	Female	23	27,04	4,248	,886
PSS_after	Male	13	13,31	5,023	1,393
	Female	23	16,96	4,084	,852
cortisol_level1	Male	13	,82962	,597452	,165703
	Female	22	,86536	,487868	,104014
cortisol_level2	Male	12	,65025	,380993	,109983
	Female	22	,54600	,187896	,040059
BMI_Bef	Male	13	23,6545	2,40731	,66767
	Female	23	28,2194	3,81263	,79499
BMI_Af	Male	13	23,6136	2,44965	,67941
	Female	23	28,1596	3,80261	,79290

N-sample size. Std- standard deviation. PANSS- positive and negative symptoms scale. PSS- Perceived Stress Scale. BMI- body mass index.

The results of the descriptive statistics (Table 6) reveal that females display higher mean ranges of age, PANSS After ECT, PSS before ECT, PSS after ECT, cortisol levels after and before ECT, BMI after and BMI before ECT.

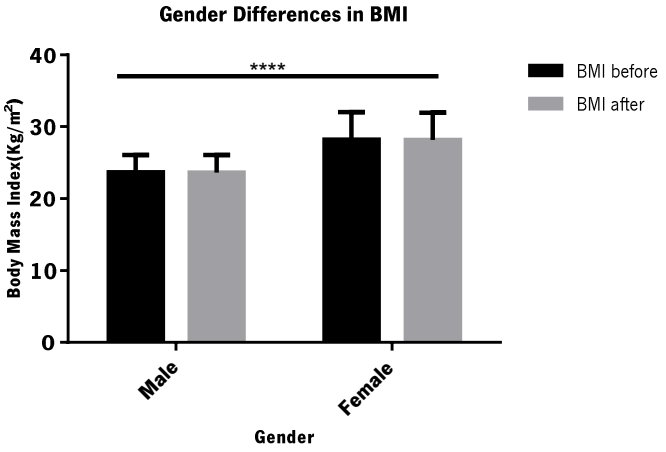
Table 7. Independent t-test differences between gender

Variable measured		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
age	Equal variances assumed	-2,566	34	,015	-12,508	4,876	-22,417	-2,600
PANSSafter	Equal variances assumed	-2,196	34	,035	-10,431	4,750	-20,084	-,778
PSS_befor	Equal variances assumed	-1,901	34	,066	-3,582	1,884	-7,410	,247
PSS_after	Equal variances assumed	-2,369	34	,024	-3,649	1,540	-6,779	-,519
cortisol_level1	Equal variances assumed	-,193	33	,848	-,035748	,185527	-,413207	,341710

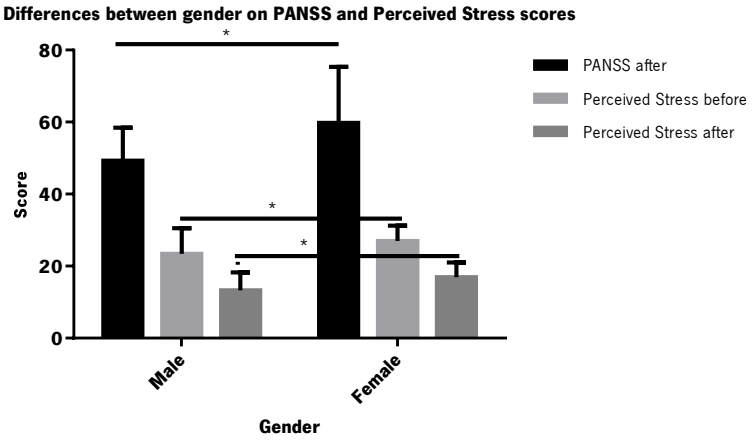
cortisol_level2	Equal variances assumed	1,075	32	,291	,104250	,097005	-,093343	,301843
BMI_Bef	Equal variances assumed	-3,888	34	,000	-4,56494	1,17419	-6,95119	-2,17870
BMI_Af	Equal variances assumed	-3,868	34	,000	-4,54600	1,17538	-6,93466	-2,15734

Significant level. p<0,05

There were statistically significant differences between males and females in the following variables: age t(34)=-2,566 p=0,015 medium effect size r=0,4, PANSS after ECT t(34)=-2,196 p=0,035 medium effect size r=0,35, PSS after ECT t(34)=-2,369 p=0,024 medium effect size r=0,38, body mass index(BMI) before ECT t(34)=-3,888 p<0,001 large effect size r=0,55 and BMI after ECT t(34)=-3,868 p<0,001. There were no statistically significant difference between male and female in the following variables: PSS before ECT t(34)=-1,901 p=0,66, Salivary cortisol before ECT t(33)=-0,193 p=0,848 and salivary sortisol after ECT t(32)=0,291 p=0,291.



9. Differences between genders in BMI

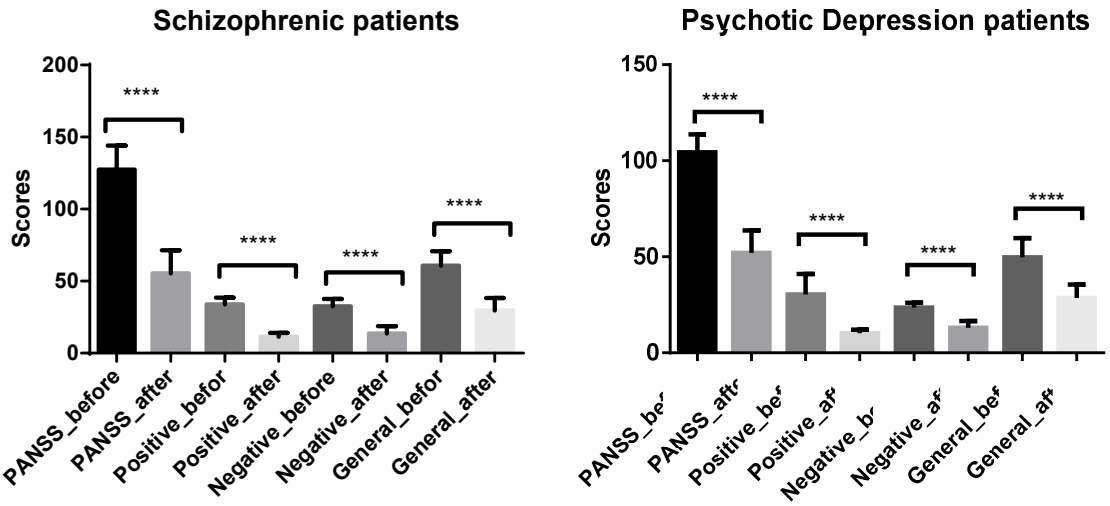


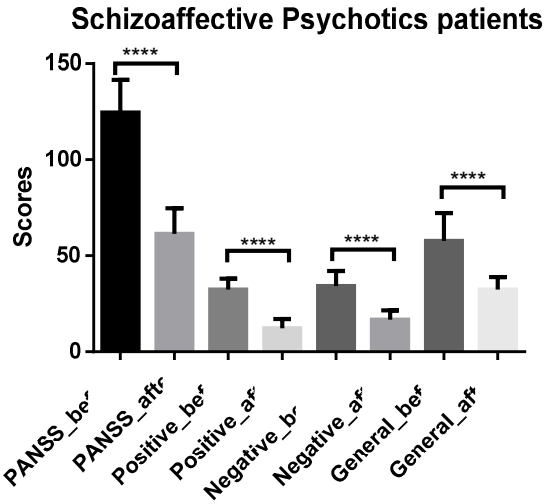
10. Differences between genders on PANSS. *p value less than 0,05.

4.2.1. IMPROVEMENT OF NEGATIVE, POSITIVE AND GENERAL SYMPTOMS AFTER ECT

The first goal of our work was to evaluate the evolution of positive and negative symptoms in psychotic patients undergoing ECT. For this aim, patients were evaluated by a psychiatrist with the use of PANSS scale before the first ECT, and one week after the last ECT. As expected, the patients shown decrease in PANSS scores which is in line with the improvement of positive, negative and general symptoms. The psychotic depression group were those whom shown more clinical improvement after ECT.

A) B)



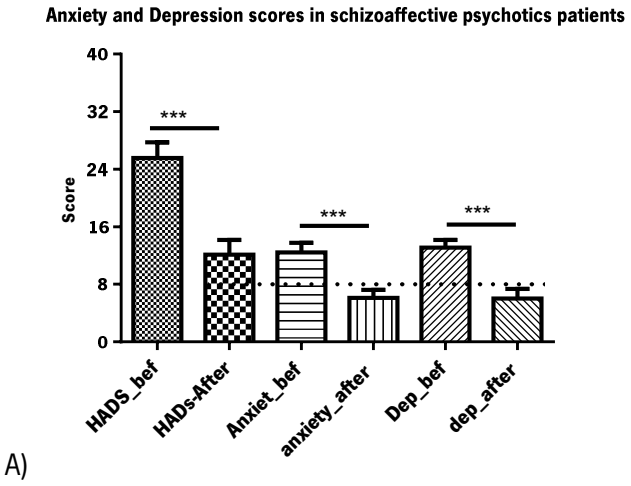


C)

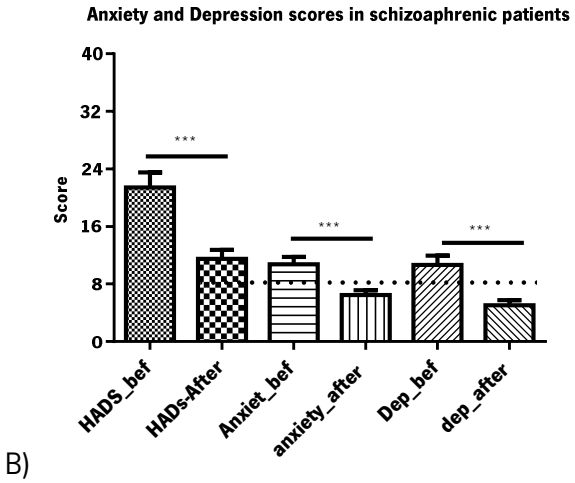
Figure 11 Measure of PANSS before and after ECT in three different psychotic inpatients groups: Schizophrenic patients (A), Psychotic Depression (B) and Schizoaffective Psychosis (C). In all of them the analysis was performed using dependent t-Test. Bars mean group scores before and after ECT (t-test, "" p<0,001).

4.2.2. ANXIETY AND DEPRESSION AMONG PSYCHOTICS PATIENTS

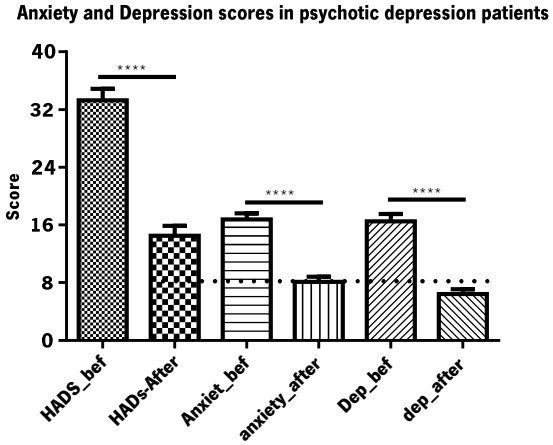
Results of Hospital Anxiety and Depression scale revealed decrease of anxiety and depression scores in all three patients groups a week after ECT. With the cut-off point of 8, Psychotic depression shown the less reduction of anxiety score, despite the fact that all of them improves anxiety after ECT.



A)



B)



C)

Figure 12 Measure of Anxiety and Depression perceived before and after ECT in three different psychotic inpatients groups: (A) Schizoaffective Psychosis (n=9), (B)Schizophrenic patients (n=17) and (C)Psychotic

Depression (10). In all of them the analysis was performed using dependent t-Test. Bars - mean of group scores before and after ECT (dependent t-test, ⁻⁻⁻⁻ p<0,001, ⁻⁻⁻ p<0,01).

4.2.3. PERCEIVED STRESS

Our data reveals an improvement of perceived stress after ECT in all psychotic patients' groups. Psychotic depression patients displayed the higher improvement in perceived stress taking into account that they displayed higher levels of perceived stress before treatment (Figure 13).

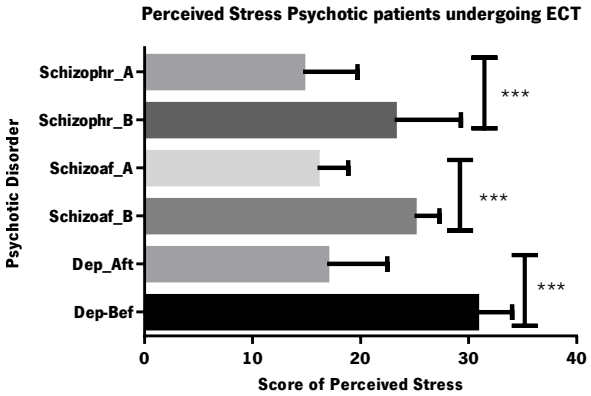


Figure 13Perceived stress among patients undergoing ECT in three group's diagnosis, after and before ECT. ⁻⁻⁻p<0,001.

4.2.4. CORTISOL AMONG PSYCHOTICS PATIENTS UNDERGOING ECT

The specific analysis of the variations of the salivary cortisol levels in each experimental group using the dependent t-test revealed that patients with schizophrenia and psychotic depression displayed a significant decrease in this measure after ECT. No significant differences were observed in schizoaffective psychotic patients. (Figure 14)

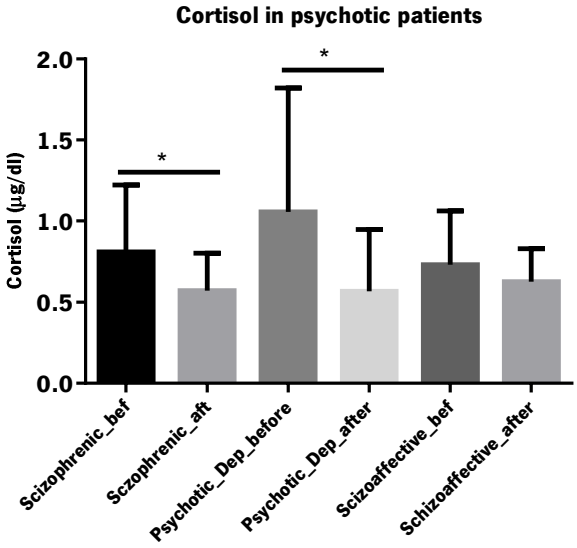


Figure 14 Cortisol in three psychotic patients groups before and after ECT. ·p<0,05.

4.3. CORRELATION BETWEEN VARIABLES

Table 8. General correlations

		age	Ed. Lev	PANSS_B	PANSS_A	PSS_B	PSS_A	HADS_B	HADS_A	Cortisol_B	Cortisol_A	Duration_disord	Percent of resp
Age	r		-,544··	,050	,309	,490··	,395·	,612··	,531··	,238	,014	,645··	,314
Ed-Lev	r	-,544··		-,026	-,139	-,287	,052	-,270	-,354·	-,081	,047	-,397·	-,142
PANSS_B	r	,050	-,026		,557··	-,256	,133	-,157	-,053	,089	,112	,019	-,039
PANSS_A	r	,309	-,139	,557··		,204	,446··	,239	,377·	,115	-,021	,036	,797··
PSS_B	r	,490··	-,287	-,256	,204		,541··	,727··	,529··	,135	-,227	,298	,433··
PSS_A	r	,395·	,052	,133	,446··	,541··		,507··	,471··	,299	,203	,112	,462··
HADS_B	r	,612··	-,270	-,157	,239	,727··	,507··		,584··	,062	-,150	,184	,391·
HADS_A	r	,531··	-,354·	-,053	,377·	,529··	,471··	,584··		,102	-,037	,099	,475··
Cortisol_B	r	,238	-,081	,089	,115	,135	,299	,062	,102		,615··	,137	,083
Cortisol_A	r	,014	,047	,112	-,021	-,227	,203	-,150	-,037	,615··		-,037	-,076
Duration_disord	r	,645··	-,397·	,019	,036	,298	,112	,184	,099	,137	-,037		,028
Percent of resp	r	,314	-,142	-,039	,797··	,433··	,462··	,391·	,475··	,083	-,076	,028	

r-Pearson coefficient correlation. *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). Statistically significant correlations are highlighted in the table.

To determine if there were correlations between variables among all the psychotic patients undergoing ECT, we carried out Pearson correlation analysis. Our results show that age was negatively correlated with education level (r=-0,544, p<0.05), and positively correlated with perceived stress before ECT(r=0,490, p<0,05), perceived stress after ECT(r=0,395, p<0,05), HADS before ECT(r=0,612, p<0,05), and HADS after ECT (r=0,531, p<0,05). Education level was negatively correlated with HADS after ECT (r=-0,354 p<0,05). PANSS after ECT was positively correlated with perceived stress after ECT (r=0,446 p<0,05) and HADS after ECT (r=0,377 p<0,05). HADS before ECT was positively correlated with perceived stress after ECT (r=0,507 p<0,05) and percentage of response to ECT (r=0,391 p<0,05). HADS after ECT was positively correlated with perceived stress before ECT (r=0,529 p<0,05) and perceived stress after ECT (r=0,471 p<0,05).

4.3.1. CORRELATION BETWEEN VARIABLES AMONG SCHIZOPHRENIC PATIENTS

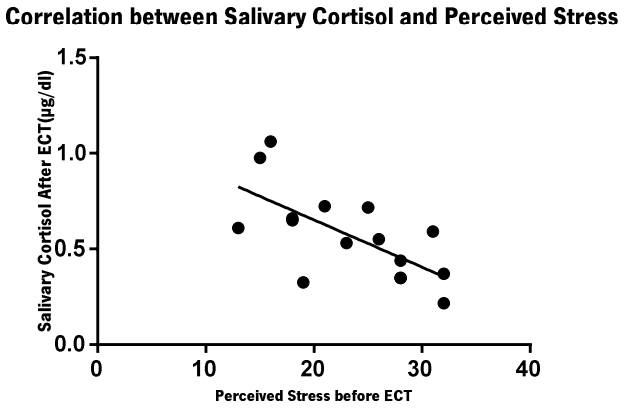
Table 9. Correlations in schizophrenic patients

		age	Ed_Lev	PANSS_B	PANSS_A	PSS_B	PSS_A	HADS_B	HADS_A	Cortisol_B	Cortisol_A	Percent	Negative_A
age	r		-,550*	0,392	,531*	,490*	,536*	,753**	,568*	0,22	-0,09	0,448	,622**
Ed_Lev	r	-,550*		-0,06	-0,195	-,527*	0,005	-,644**	-,509*	0,23	,509*	-0,234	-0,239
PANSS_B	r	0,392	-0,06		,731**	0,06	,491*	0,413	0,25	0,48	0,139	0,341	,685**
PANSS_A	r	,531*	-0,2	,731**		0,405	,616**	,541*	,612**	0,27	-0,21	,884**	,951**
PSS_B	r	,490*	-,527*	0,06	0,405		,510*	,625**	,578*	-0,2	-,670**	,495*	,487*
PSS_A	r	,536*	0,01	,491*	,616**	,510*		,529*	0,472	0,26	-0,2	,509*	,589*
HADS_B	r	,753**	-,644**	0,413	,541*	,625**	,529*		,670**	-0,1	-0,38	0,445	,609**
HADS_A	r	,568*	-,509*	0,25	,612**	,578*	0,472	,670**		-0,3	-,554*	,652**	,613**

Cortisol_B	r	0,223	0,23	0,479	0,266	-0,183	0,258	-0,055	-0,254		0,456	0,049	0,239
Cortisol_A	r	-0,088	,509*	0,139	-0,213	-,670**	-0,2	-0,375	-,554*	0,46		-0,39	-0,271
Percent	r	0,448	-0,23	0,341	,884**	,495*	,509*	0,445	,652**	0,05	-0,39		,822**
Negative_A	r	,622**	-0,24	,685**	,951**	,487*	,589*	,609**	,613**	0,24	-0,27	,822**	

r-Pearson coefficient correlation. *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). Statistically significant correlations are highlighted in the table.

We have carry out Pearson correlation analysis between schizophrenic patients to see if there is any correlation between variables in this specific group.



.**Figure 15.Correlation between salivary cortisol and perceived stress before ECT.**

A negative correlation between salivary cortisol levels after ECT and perceived stress before ECT was found in schizophrenic patients (r=-0,670 p<0,001). Patients with higher perceived stress before ECT showed lower salivary cortisol levels after ECT (Figure 15).

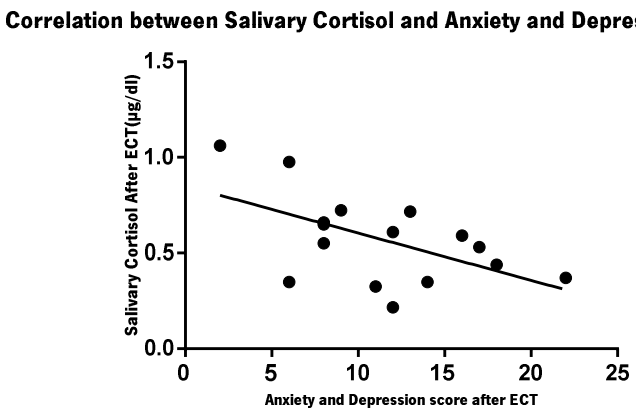
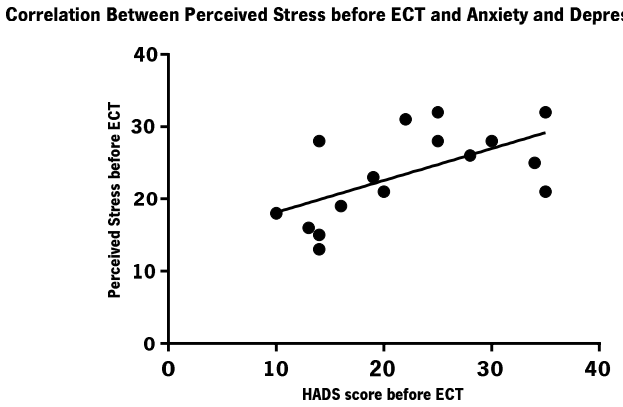
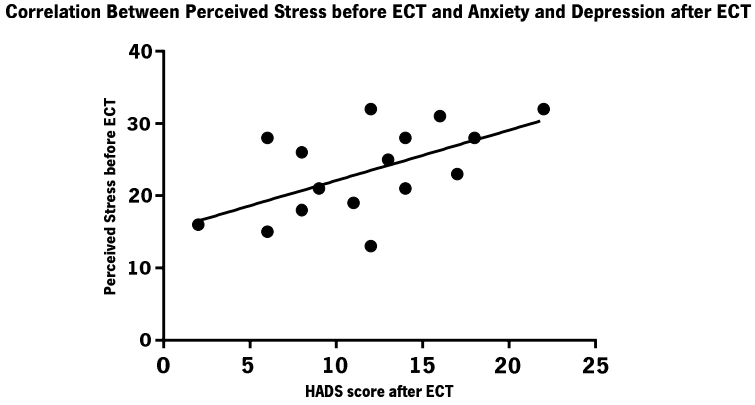


Figure 16.Correlation between salivary cortisol after ECT and HADS score after ECT

A negative correlation between salivary cortisol after ECT and anxiety and depression scores after ECT(r=-0,554 p<0,05) was determined. Schizophrenic patients with higher scores of anxiety and depression after ECT displayed lower salivary cortisol levels after ECT (Figure 16).



A)



B)

Figure 17.Correlation between perceived stress and anxiety and depression in schizophrenic patients Fig(A)- Correlation between perceived stress before ECT and HADS before ECT, Fig(B)- Correlation between perceived stress before ECT and HADS after ECT.

Perceived stress before ECT was positively correlated with anxiety and depression before ECT(r= 0,625 p<0,001) and anxiety and depression after ECT (r=0,578 p<0,05). Schizophrenic patients with higher perceived stress before ECT, showed higher scores of anxiety and depression in the two time points (Figure 17).

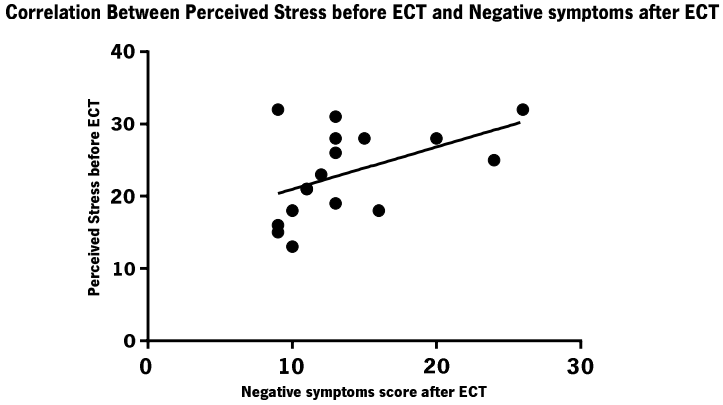


Figure 18. Correlation between perceived stress after ECT and negative symptoms

Perceived stress was positively correlated with negative symptoms after ECT in schizophrenic patients (r=0,487 p<0,05). Patients with higher perceived stress before ECT displayed higher negative symptoms after ECT (Figure 18).

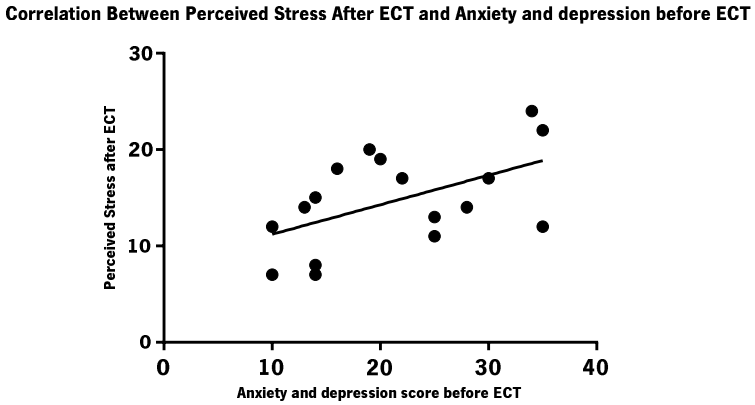


Figure 19.Correlation between perceived stress after ECT and anxiety and depression before ECT

Perceived stress after ECT were positively correlated with anxiety and depression before ECT(r=0,529 p<0,05). Schizophrenic patients with higher levels of anxiety and depression before ECT displayed higher levels of perceived stress after ECT (Figure 19).

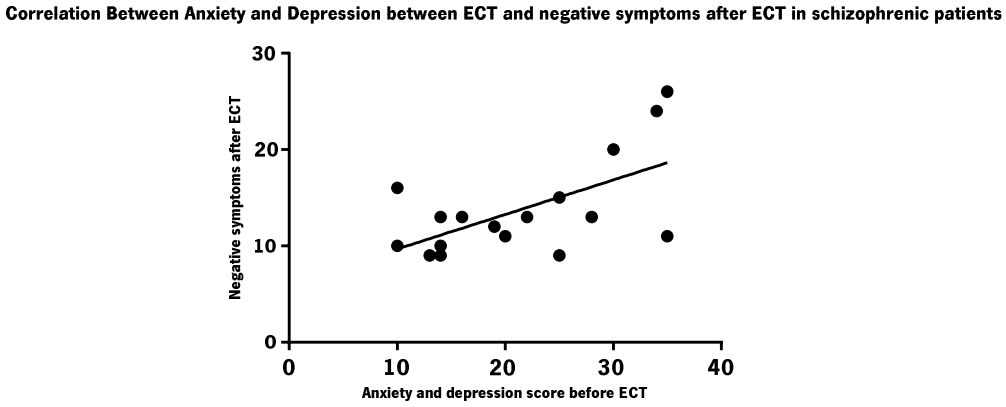


Figure 20. Correlation between negative symptoms after ECT and depression after ECT

Negative symptoms after ECT were positively correlated with anxiety and depression scores before ECT in schizophrenic patients (r=0,609 p<0,01). Schizophrenic patients with higher scores of anxiety and depression after ECT displayed higher negative symptoms after ECT (Figure 20).

4.3.2. CORRELATION BETWEEN VARIABLES IN PSYCHOTIC DEPRESSION PATIENTS GROUP

Table 10. Correlation in psychotic depression patients

		Age	Positive symptoms Before	PSS Before	HADS After	Anxiety After	Depression After	Cortisol Before
Age	r		,502	,126	,282	,258	,283	,421
PANSSbefore	r	,681*	,399	,238	,263	,206	,298	,279
negative_symptomsbef	r	,722*	,266	-,098	-,228	-,273	-,165	-,120
PSS_before	r	,126	-,104		,820**	,774**	,801**	,363
anxiety_score	r	-,419	-,746*	,284	,069	,080	,052	,006

depression_score	r	,230	-,336	,694*	,537	,403	,628	,186
HADS_after	r	,282	,141	,820**		,960**	,961**	,609
anxiety_scoreafter	r	,258	,151	,774**	,960**		,846**	,632*
depression_scoreafter	r	,283	,121	,801**	,961**	,846**		,539
cortisol_level1	r	,421	,311	,363	,609	,632*	,539	

r-Pearson coefficient correlation. *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

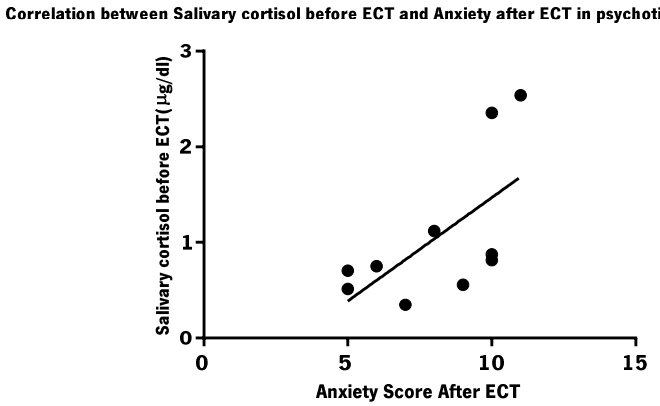
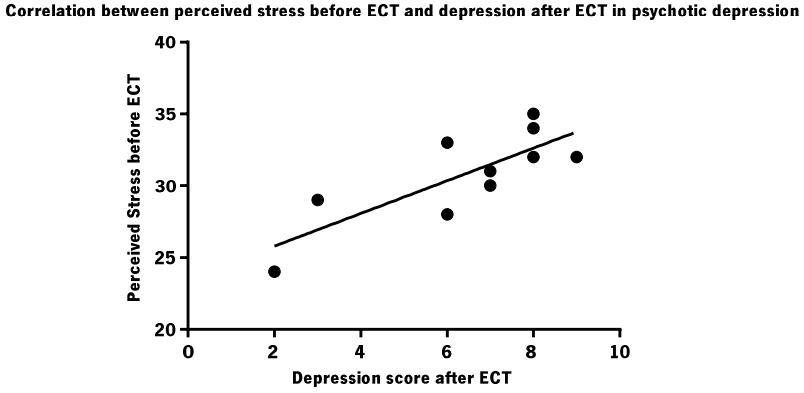


Figure 21. Correlation between salivary cortisol and anxiety score after ECT

Salivary cortisol in psychotic depression patients were positively correlated with anxiety scores after ECT (r=0,632 p<0,05)(Figure21)



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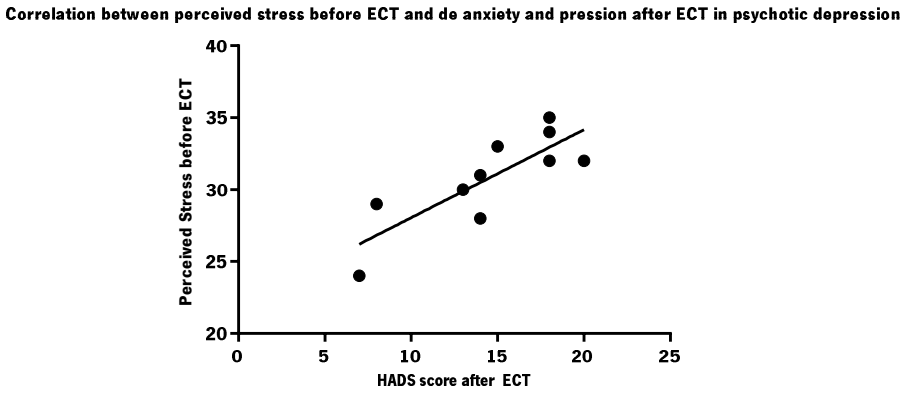
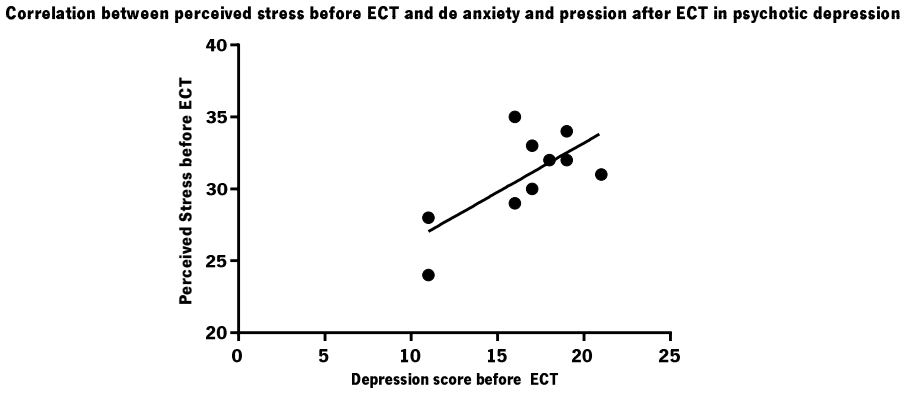


B)



C)

Figure 22. Correlation between perceived stress and other variables Fig(A)- Correlation between perceived stress before ECT and depression score after ECT. Fig.(B)- Correlation between perceived stress before ECT and HADS after ECT. Fig.(C)- Correlation between perceived stress before ECT and depression score before ECT.

Perceived stress in psychotic depression patients was positively correlated with depression before ECT(r=0,694 p<0,05), depression after ECT (r=0,801 p<0,01) and HADS after ECT (r=0,820 p<0,01)(Figure 22).

4.3.3. CORRELATION BETWEEN VARIABLES IN SCHIZOAFFECTIVE PSYCHOTIC PATIENTS GROUP

Table 11. Correlation in schizoaffective patients

		Age	Lev_Ed.	PANNS_B	PSS_B	HADS_A	Cortisol_B	Cortisol_A	negative_symptomsbef
age	r		-,756*	,681*	,126	,282	,421	,064	,722*
scholarity	r	-,756*		-,479	,412	,196	-,251	,009	-,676*
PANSSbefore	r	,681*	-,479		,238	,263	,279	,102	,720*
PSS_befor	r	,126	,412	,238		,820**	,363	,237	-,098
HADS_after	r	,282	,196	,263	,820**		,609	,541	-,228
cortisol_level1	r	,421	-,251	,279	,363	,609		,790**	-,120
cortisol_level2	r	,064	,009	,102	,237	,541	,790**		-,327
negative_symptomsbef	r	,722*	-,676*	,720*	-,098	-,228	-,120	-,327	

r-Pearson coefficient correlation. *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

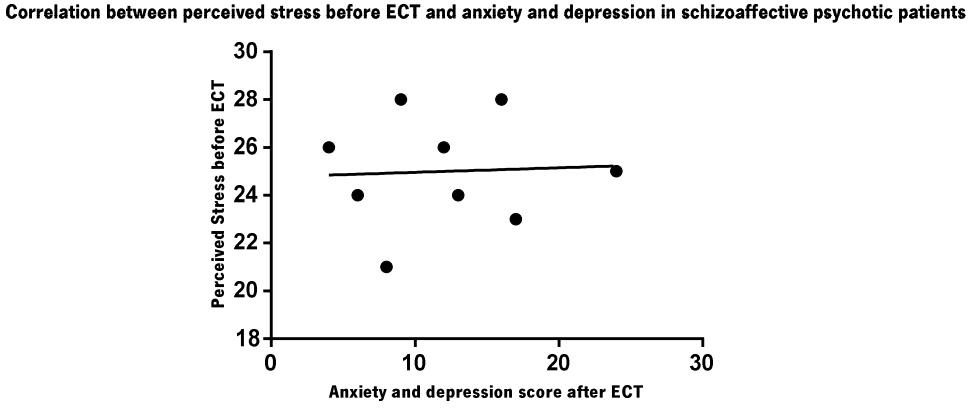


Figure 23. Correlation between perceived stress before ECT and anxiety and depression after ECT

Perceived stress before ECT was positively correlated with anxiety and depression after ECT in schizoaffective psychotic patients (r=0,820 p<0,01). Patients who presented higher scores of perceived stress before ECT showed higher levels of anxiety and depression after ECT (Figure 23).

4.4. MODEL REGRESSION TO PREDICT CLINICAL OUTCOME IN PSYCHOTIC PATIENTS UNDERGOING ECT

To predict which variables may predict the clinical outcome after ECT treatment, we performed a simple regression analysis with two predictor variables.

4.4.1. ANXIETY AND DEPRESSION BEFORE ECT AS A PREDICTOR OF RESPONSE TO ECT IN PSYCHOTIC PATIENTS.

Table 12. HADS model regression

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	,391[a]	,153	,128	9,20199	1,473

a. Predictors: (Constant), HADS_before

The value of R square of 0,153, shows that HADS can account for 15,3% of the variation in rate of response to ECT in psychotic patients.

Table 13. ANOVA HADS

	Model	Sum of Squares	df	Mean Square	F	Sig.
1	Regression	520,470	1	520,470	6,147	,018[b]
	Residual	2879,003	34	84,677		
	Total	3399,474	35			

Significant level p<0,05

Table 14. Coefficients of regression

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95,0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	35,226	4,838		7,281	,000	25,394	45,058
	HADS_before	,442	,178	,391	2,479	,018	,080	,804

Significant level p<0,05

By analysis of ANOVA table (table 13), we can conclude that our regression model predicts the rate of response to ECT in psychotic patients. If our predictor variable (HADS before ECT) increased by one unit, our model predicts that 0,442 percent of response to ECT will increase.

Our equation model is : Response to ECT=35,226+(0,442*HADS level before ECT)

4.4.2. PERCEIVED STRESS BEFORE ECT AS A GOOD PREDICTOR OF RESPONSE TO ECT IN PSYCHOTIC PATIENTS

Table 15. PSS Model

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	,433[a]	,188	,164	9,01155	1,494

a. Predictors: (Constant), PSS_befor

The value of R square of 0,188, shows that perceived stress before ECT can account for 18,8% of the variation in the rate of response to ECT in psychotic patients.

Table 16.ANOVA PSS

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	638,403	1	638,403	7,861	,008[b]
	Residual	2761,071	34	81,208		
	Total	3399,474	35			

****p value<0,05.**

Table 17. Coefficient of regression model

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95,0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	27,063	7,129		3,796	,001	12,575	41,550
	PSS_befor	,759	,271	,433	2,804	,008	,209	1,309

****p value<0,05.**

By analysis of the ANOVA table (table 16), we conclude that our regression model predicts the rate of response to ECT in psychotic patients. If our predictor variable (perceived stress before ECT) increased by one unit, our model predicts that 0,759 percent of response to ECT will increase.

Our equation model is : Response to ECT=27,063+(0,759*Perceived stress level before ect)

5. DISCUSSION AND CONCLUSION

5.1. CLINICAL AND SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PATIENTS

The population of psychotic patients studied had a mean age of 49 years old, indicating that this group was composed of chronic psychotic patients, all of them with poor response to antipsychotic drugs. The higher mean BMI of this cohort is probably related to its age range and illness conditions, such as long term antipsychotic treatment and poor physical exercise.

5.2. RESPONSE TO ECT IN PSYCHOTIC PATIENTS

In general, all groups showed a good response to ECT. Previous studies have classified as responders the patients who show a reduction in the PANSS total score higher than 50% after ECT (Schennach-Wolff et al., 2011). In our study, a significant number of patients met the criteria of responders. Furthermore, our psychotic depression patients showed better response to ECT which is in line with a previous data (Anthony J. Rothschild, 2003).

5.3. PSYCHOMETRIC MEASUREMENTS OF CLINICAL IMPROVEMENT AFTER ECT

In this study we were able to show that ECT improves clinical outcomes in all psychotic patients. The psychometrics variables were assessed using PANSS scale, Portuguese version of PSS scale and HADS scale. Data of all three items of PANSS, revealed a significant decrease after ECT in all psychotic patients' groups. These findings are supported by other studies (Garg, Chavan, & Arun, 2011). The effectiveness of ECT was different between psychotic depression patients and schizophrenic patients, with not statistically significant differences between these groups and schizoaffective patients. These differences might be explained by the fact that schizophrenic and schizoaffective patients showed higher scores of negative symptoms, which remain resistant to the present antipsychotics pharmacological approaches.

Perceived stress was improved in all psychotic patients groups after EC., This finding is in line with previous studies and may be explained by the increase of stress in psychotic patients before admission and decrease of perceived stress during inpatient treatment All psychotic patients revealed improvement in anxiety and depression after ECT, which is in line with a previous study (Vukadin et al., 2011).

5.4. THE EFFECT OF ECT IN THE HPA- AXIS

Additionally, we explored the effect of this non-pharmacological biological treatment in the HPA axis function, namely by measuring the levels of salivary cortisol in two time points and correlating with perceived stress and clinical outcome. We were able to demonstrate the main biological impact of ECT in the improvement of clinical symptoms in psychotic patients resistant to antipsychotic treatment. Psychotic depression and schizophrenic patients showed a decrease in salivary cortisol after ECT. This may be explained by the fact that an impairment of HPA axis in psychotic patients has been described (Vukadin et al., 2011),(Yeap & Thakore, 2005). We can conclude that the ECT is associated with transient neuro-endocrine changes, and that the ECT does not harm normal neuro-endocrine communication and function in these psychotic patients which is in line with the findings of study (Fluitman et al., 2011).

5.5. CORRELATIONS BETWEEN CORTISOL AND CLINICAL VARIABLES

Schizophrenic patients revealed a negative correlation between salivary cortisol after ECT with perceived stress before ECT and anxiety after ECT. This findings suggests that schizophrenic patients who had higher scores of perceived stress before ECT may have poor clinical outcome with disturbance of mood domain. On the other hand, salivary cortisol before ECT was positively correlated with anxiety after ECT in psychotic depression patients group. This finding supports previous studies that suggest an impairment of HPA axis in psychotic depression. Cortisol deregulation may impair the mechanisms of arousal and cognitive pathways and this may be the backbone of mood impairment in these psychotic patients. Our study shows that ECT may have a minimal effect on the regulation of the HPA-axis in psychotic depression and schizophrenic patients, despite the fact that no effect was observed in schizoaffective patients.

5.6. CORRELATIONS BETWEEN VARIABLES,AND REGRESSION MODEL

By analysis of all experimental groups we found that HADS score before ECT was positively correlated with perceived stress after ECT. Perceived stress before ECT was statistically correlated with anxiety and depression before ECT, anxiety and depression after ECT, negative symptoms after ECT in schizophrenic patient. In this psychotic group also we found correlation between perceived stress after ECT and anxiety and depression before ECT. Perceived stress before ECT were also correlated with depression after ECT, anxiety and depression after ECT and

depression before ECT in psychotic depression group of patients. Schizoaffective group patients revealed positive statistically significant correlation between perceived stress and anxiety and depression after ECT.

Importantly we carried out a regression model to find good predictor variables of response to ECT. We provided the first demonstration that perceived stress and anxiety and depression score are good predictors of the clinical outcome in these three chronic psychotic patients groups.

5.7. CONCLUSION:

The results of our study suggest that ECT is effective and a valuable therapeutic option which could be useful as adjunctive therapy in psychotic patient's refractory to antipsychotic drugs including clozapine. There was a noticeable improvement in the PANSS, HADS and PSS scores after ECT. This study also revealed an evidence of ECT use in chronic psychotic patients, while most studies were carried out in first psychotic episode. The decrease of salivary cortisol after ECT in our patients study may suggest that ECT has an important effect in HPA axis, improving its function. Whilst the mechanism by how it can improve HPA axis is unclear, it become clear according to our results that the correlation between HADS score and ratio of response to ECT, showed us that those patients with higher HADS scores before ECT and negative symptoms might have higher benefits with early treatment with ECT.

5.8. LIMITATIONS AND FUTURE DIRECTIONS:

The most important limitations of this study were the reduced number of patients and the lack of a control group and randomization of the sample. For future studies, we propose to design approaches in animal models of ECT and psychotic disorder. On the other hand, it would be useful to design longitudinal studies with more patients and long term follow-up studies with epigenetic approaches and correlations of ECT with other adjunctive therapy such as cognitive behavior therapy and neuro-feedback therapy.

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7. ATTACHMENTS

Número aleatório

Consentimento Informado

Considerando a "Declaração de Helsínquia" da Associação Médica Mundial
(Helsínquia 1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996 e Edimburgo 2000)

Caro paciente,
Convidamo-lo a participar num estudo observacional, na prática clínica habitual, sobre o efeito da electroconvulsivoterapia no controlo da sintomatologia delirante, coordenado pelo Prof. Doutor João Bessa, Psiquiatra no Hospital de Braga e no Instituto de Investigação em Ciências da Vida e da Saúde (ICVS) da Escola de Ciências da Saúde da Universidade do Minho e pelo Professor Doutor António Pacheco Palha, Psiquiatra e Director Clínico da Casa de Saúde do Bom Jesus – Braga - e Professor Catedrático de Psiquiatria na Universidade do Porto.

O objetivo deste estudo é saber se os utentes submetidos a electroconvulsivoterapia mostram melhoria dos sintomas delirantes e a relação do quadro clinico com os niveis de stress e de cortisol salivar.

Caso concorde em participar neste estudo, devidamente autorizado pela comissão de ética do seu hospital, recolheremos apenas informação estritamente necessária sobre a sua situação clínica atual, incluindo o diagnóstico, evolução da doença, recolheremos também amostras de saliva antes e após o término das sessões de electroconvulsivoterapia.

Os seus dados clínicos e a amostra de saliva serão conservados, em condições adequadas e sem elementos que o permitam identificar, nas instalações do Instituto de Investigação em Ciências da Vida e da Saúde da Universidade do Minho e poderão ser utilizados, de forma anónima, noutros estudos, exclusivamente com fins de investigação, desde que devidamente autorizados por uma Comissão de Ética.

Caso prefira não participar, os procedimentos diagnósticos e terapêuticos devidos à sua situação actual não serão minimamente afectados e continuará a ser seguido pelo seu psiquiatra assistente. Se tiver alguma dúvida em relação a este estudo, por favor coloque-a ao seu médico assistente até ficar completamente esclarecido.

Muito obrigado pela sua atenção!

ACEITAÇÃO

Declaro que fui informado do estudo" Efeito da Electroconvulsivoterepia no controlo da sintomatologia delirante em pacientes com esquizofrenia" pelo Dr _____, que respondeu a todas as minhas questões.

Declaro também que aceito participar no mesmo estudo, autorizando a recolha de dados sobre a minha situação clínica actual, a colheita e armazenamento de amostras de saliva, bem como a sua utilização futura, exclusivamente para fins de investigação.

Data e local: _____

O paciente: _____

Assinatura: _____

O médico: _____

Assinatura:_____

Os Orientadores:

_____

(Prof.Doutor João Bessa)

_____

(Professor Doutor António Pacheco Palha)

ESCALA DE ANSIEDADE E DEPRESSÃO HOSPITALAR

Este questionário foi construído para ajudar a saber como se sente. Pedimos-lhe que leia cada uma das perguntas e faça uma cruz (X) no espaço anterior à resposta que melhor descreve a forma como se tem sentido na última semana. Não demore muito tempo a pensar nas respostas. A sua reação imediata a cada questão será provavelmente mais correta do que uma resposta muito ponderada. Por favor, faça apenas uma cruz em cada pergunta.

1. Sinto-me tenso (a) ou nervoso (a):
( ) Quase sempre
( ) Muitas vezes
( ) Por vezes
( ) Nunca

2. Ainda sinto prazer nas coisas de que costumava gostar:
( ) Tanto como antes
( ) Não tanto agora
( ) Só um pouco
( ) Quase nada

3. Tenho uma sensação de medo, como se algo terrível estivesse para acontecer:
( ) Sim e muito forte
( ) Sim, mas não muito forte
( ) Um pouco, mas não me aflige
( ) De modo algum

4. Sou capaz de rir e ver o lado divertido das coisas:
( ) Tanto como antes
( ) Não tanto como antes
( ) Muito menos agora
( ) Nunca

5. Tenho a cabeça cheia de preocupações:
( ) A maior parte do tempo
( ) Muitas vezes
( ) Por vezes
( ) Quase nunca

6. Sinto-me animado (a):
( ) Nunca
( ) Poucas vezes
( ) De vez em quando
( ) Quase sempre

7. Sou capaz de estar descontraidamente sentado(a) e sentir-me relaxado(a):
( ) Quase sempre
( ) Muitas vezes
( ) Por vezes
( ) Nunca

8. Sinto-me mais lento(a), como se fizesse as coisas mais devagar:
( ) Quase sempre
( ) Muitas vezes
( ) Por vezes
( ) Nunca

9. Fico de tal forma apreensivo(a) (com medo), que até sinto um aperto no estômago:
( ) Nunca
( ) Por vezes
( ) Muitas vezes
( ) Quase sempre

10. Perdi o interesse em cuidar do meu aspecto físico:
( ) Completamente
( ) Não dou a atenção que devia
( ) Talvez cuide menos que antes
( ) Tenho o mesmo interesse de sempre

11. Sinto-me de tal forma inquieto(a) que não consigo estar parado(a):
( ) Muito
( ) Bastante
( ) Não muito
( ) Nada

12. Penso com prazer nas coisas que podem acontecer no futuro:
( ) Tanto como antes
( ) Não tanto como antes
( ) Bastante menos agora
( ) Quase nunca

13. De repente, tenho sensações de pânico:
( ) Muitas vezes
( ) Bastantes vezes
( ) Por vezes
( ) Nunca

14. Sou capaz de apreciar um bom livro ou um programa de rádio ou televisão:
( ) Muitas vezes
( ) De vez em quando
( ) Poucas vezes
( ) Quase nunca

Anexo 1

Escala das Síndromes Positiva e Negativa – PANSS

Escala Positiva

P1 - Delírios .....1..... 2..... 3..... 4..... 5..... 6..... 7

P2 - Desorganização conceitual .....1..... 2..... 3..... 4..... 5..... 6..... 7

P3 - Comportamento alucinatório .....1..... 2..... 3..... 4..... 5..... 6..... 7

P4 - Excitação .....1..... 2..... 3..... 4..... 5..... 6..... 7

P5 - Grandeza.....1..... 2..... 3..... 4..... 5..... 6..... 7.

P6 - Desc2onfiança .....1..... 2..... 3..... 4..... 5..... 6..... 7

P7 - Hostilidade .....1..... 2..... 3..... 4..... 5..... 6..... 7

Escore escala positiva _____

Número de sintomas avaliados >3 _____

Escala Negativa

N1 - Afetividade embotada .....1..... 2..... 3..... 4..... 5..... 6..... 7

N2 - Retraimento emocional .....1..... 2..... 3..... 4..... 5..... 6..... 7

N3 - Contato pobre.....1..... 2..... 3..... 4..... 5..... 6..... 7

N4 - Retraimento social passivo/apático .....1..... 2..... 3..... 4..... 5..... 6..... 7

N5 - Dificuldade pensamento abstrato .....1..... 2..... 3..... 4..... 5..... 6..... 7

N6 - Falta de espontaneidade e fluência .....1..... 2..... 3..... 4..... 5..... 6..... 7

N7 - Pensamento estereotipado .....1..... 2..... 3..... 4..... 5..... 6..... 7

Escore escala negativa _____

Número de sintomas avaliados >3 _____

Escala de Psicopatologia Geral

G1 - Preocupação somática .....1..... 2..... 3..... 4..... 5..... 6..... 7

G2 - Ansiedade .....1..... 2..... 3..... 4..... 5..... 6..... 7

G3 - Culpa .....1..... 2..... 3..... 4..... 5..... 6..... 7

G4 - Tensão .....1..... 2..... 3..... 4..... 5..... 6..... 7

G5 - Maneirismo/postura .....1..... 2..... 3..... 4..... 5..... 6..... 7

G6 - Depressão .....1..... 2..... 3..... 4..... 5..... 6..... 7

G7 - Retardo motor .....1..... 2..... 3..... 4..... 5..... 6..... 7

G8 - Falta de cooperação.....1..... 2..... 3..... 4..... 5..... 6..... 7

G9 - Conteúdo incomum pensamento .....1..... 2..... 3..... 4..... 5..... 6..... 7

G10 - Desorientação .....1..... 2..... 3..... 4..... 5..... 6..... 7

G11 - Déficit atenção .....1..... 2..... 3..... 4..... 5..... 6..... 7

G12 - Juízo e crítica.....1..... 2..... 3..... 4..... 5..... 6..... 7

G13 - Distúrbio volição .....1..... 2..... 3..... 4..... 5..... 6..... 7

G14 - Mau controle impulso .....1..... 2..... 3..... 4..... 5..... 6..... 7

G15 - Preocupação .....1..... 2..... 3..... 4..... 5..... 6..... 7

G16 - Esquiva social ativa .....1..... 2..... 3..... 4..... 5..... 6..... 7

Escala de Psicopatologia Geral: _____

Tipo sintomatológico:

Positivo (3 ou mais sintomas com o escore > ou = 4 na escala positiva e menos de 3 sintomas com escore > ou = 4 na escala negativa);

Negativo (3 ou mais sintomas com o escore > ou = 4 na escala negativa e menos de 3 sintomas com escore > ou = 4 na escala positiva);

Misto (3 ou mais sintomas com escore > ou = em ambas as escalas);

Nenhum tipo (quando não se aplicam os critérios anteriores).

QUESTIONÁRIO DE AUTO-RESPOSTA - PSS-10

Cohen e Williamson (1988)

Versão portuguesa preparada por

Miguel Trigo e Danilo Silva, 2003

INSTRUÇÃO

Para cada questão, pedimos que indique **com que frequência pensou ou se sentiu de determinada maneira, durante o último mês**. Apesar de algumas perguntas serem parecidas, existem diferenças entre elas e deve responder a cada uma como uma pergunta separada. **Responda de forma rápida e espontânea**. Indique, com uma cruz (X), a alternativa que melhor se ajusta à sua situação.

	Nunca	Quase nunca	Algumas vezes	Frequentemente	Muito frequente
	0	**1**	**2**	**3**	**4**
1. No último mês, com que frequência esteve preocupado(a) por causa de alguma coisa que aconteceu inesperadamente?					
2. No último mês, com que frequência se sentiu incapaz de controlar as coisas importantes da sua vida?					
3. No último mês, com que frequência se sentiu nervoso(a) e em stresse?					
4. No último mês, com que frequência sentiu confiança na sua capacidade para enfrentar os seus problemas pessoais?					
5. No último mês, com que frequência sentiu que as coisas estavam a correr à sua maneira?					
6. No último mês, com que frequência sentiu que não aguentava com as coisas todas que tinha para fazer?					
7. No último mês, com que frequência foi capaz de controlar as suas irritações?					
8. No último mês, com que frequência sentiu ter tudo sob controlo?					
9. No último mês, com que frequência se sentiu furioso(a) por coisas que ultrapassaram o seu controlo?					
10. No último mês, com que frequência sentiu que as dificuldades se estavam a acumular tanto que não as conseguia ultrapassar?					
	0	**1**	**2**	**3**	**4**

Questionário do Estudo

1. Número de Inquérito:_____

2. Data da colheita dos dados:___/____/2014

3. Identificação do paciente:

Iniciais do nome completo do utente:_____

Idade (anos):_____

-idade do diagnóstico da Perturbação psiquiátrica:_____

Sexo:

Masculino: ☐

Feminino: ☐

Estado Civil:

Solteiro(a): ☐ Casado(a): ☐ Divorciado(a): ☐ Viuvo(a): ☐

Naturalidade:_____

Residência :_____

Profissão/ocupação:_____

Escolaridade:

Sem escolaridade

Anos de escolaridade:_____

4. Hábitos:

» Alcoolicos(gramas/dl): ☐

»tabágicos(Unidade Maço Ano): ☐

Consumo de outras drogas:Sim: ___ Não:____.Se sim, quais drogas:_____

B. Dados Clínicos

1. **Dados antropométricos:**

Antes da ECT:

Peso(Kg):___Altura(m) : _____Tensão Arterial(mmHg):_____ Frequência Cardíaca :_____

Após a ECT:

Peso(Kg):___Altura(m) : _____Tensão Arterial(mmHg):_____ Frequência Cardíaca :_____

2. **Dados clínicos:**

3. **Diagnóstico Psiquiátrico (DSMIV):_____.**

Sintomatologia antes da Electroconvulsivoterapia:

Sintomatologia antes da Electroconvulsivoterapia:

_____.

4. Acompanhamento por psicólogo:
   Sim : ☐ Não: ☐

5. Toma alguma medicação psiquiátrica:
   Sim: ☐ Não: ☐ Se sim, qual:_____.

Uso de anticonceptivos:

Sim : ☐

Qual método:_____.

Não: ☐

Resultados dos testes psicométricos e dados laboratoriais:

Tabela1. PANSS

Tempo/Teste	PANSS total	Escala Positiva	Escala negativa	Psicopatologia geral
Antes da ECT				
Depois da ECT				

Tabela2. HADS

Tempo/Teste	HADS total	Escala Ansiedade	Escala Depressão
Antes da ECT			
Depois da ECT			

Tabela3. PSS

Teste	Antes da ECT	Depois da ECT
PSS-10		

Tabela4: Dados laboratoriais

Teste laboratorial	Antes da ECT	Depois da ECT
Cortisol Salivar		

Instruções de Utilização

Cortisol ELISA

Ensaio imunoenzimático para o *diagnóstico in-vitro* para a determinação quantitativa do cortisol livre na saliva humana e do total de cortisol em soro diluído.

REF **RE52611**

Σ **96**

2-8 °C

EU: IVD CE U.S.: *For in-vitro diagnostic use only.*

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1. APLICAÇÕES

Ensaio imunoenzimático para o *diagnóstico in-vitro* para a determinação quantitativa do cortisol livre na saliva humana e do total de cortisol em soro diluído como um auxiliar na avaliação da Síndrome de Cushing e doença de Addison.

2. SUMÁRIO E EXPLICAÇÃO

O cortisol (hidrocortisona, composto F) é o principal glucocorticóide nos humanos e é produzido na zona fasciculada do córtex supra-renal. 90% do cortisol em circulação está ligado a globulina de ligação aos corticóides (CBG, transcortina), cerca de 7% está ligado à albumina e apenas 1 – 3% não está ligado. Apenas a última parte representa a forma activa do cortisol.
O cortisol livre é libertado na saliva e é excretado pelos rins como uma pequena parte entre os metabólitos do cortisol. O nível de cortisol livre no sangue regula principalmente a sua secreção no córtex supra-renal num mecanismo de feedback negativo através da CRH (hormona libertadora da corticotropina) na região hipotalâmica e da ACTH na glândula pituitária, mas também é afectado por diferentes situações, sobretudo pelo stress.
Nos seres humanos, há uma flutuação fisiológica de cortisol que atinge o seu nível mais elevado de manhã e o mais baixo a meio da noite. Esta flutuação no nível de plasma do cortisol reflecte-se na saliva normalmente atingindo um pico nos primeiros 90 minutos depois de acordar.
A medição de cortisol está indicada para doenças com produção anormal de glucocorticóides, como por exemplo na Síndrome de Cushing e doença de Addison. Devido à flutuação diurna dos níveis de cortisol, é necessário tirar várias amostras para fazer um perfil individual de cortisol ou durante a realização de testes dinâmicos, como o teste de supressão pela dexametasona ou o teste de estimulação com ACTH. Portanto, a recolha de uma amostra de saliva é um método fácil sem a pressão das repetidas punções venosas. A medição do cortisol na saliva é aconselhável em doentes com níveis anormais de CBG, tais como mulheres grávidas, pessoas com hipotiroidismo, síndrome nefrótica ou obesidade acentuada, e durante a utilização de diferentes medicamentos, incluindo contraceptivos orais.

3. PRINCÍPIO DO TESTE

Enzyme-Linked ImmunoSorbent Assay (ELISA) em fase sólida baseado no princípio de competição. Uma quantidade indeterminada de antigénio presente na amostra e uma quantidade fixa de antigénio marcado com enzima competem pelos locais de ligação dos anticorpos que revestem os poços. Depois da incubação, os poços são lavados para parar a reacção de competição. Após a reacção do substrato, a intensidade da cor desenvolvida é inversamente proporcional à quantidade de antigénio na amostra. Os resultados das amostras podem ser determinados directamente usando a curva padrão.

4. AVISOS E PRECAUÇÕES

1. Apenas para diagnóstico *in vitro*. Apenas para utilização profissional.
2. Antes de iniciar o teste, leia as instruções completa e cuidadosamente. Utilize a versão válida do folheto informativo fornecido com o kit. Tenha a certeza de ter entendido tudo.
3. Em caso de danos no kit por favor contacte a IBL ou o seu fornecedor por escrito, até uma semana após ter recebido o kit. Não utilize componentes danificados na execução do teste, mas guarde-os para reclamação.
4. Obedeça ao número de lote e ao prazo de validade. Não misture reagentes de diferentes lotes. Não utilize reagentes expirados.
5. Siga as boas práticas de laboratório e as normas de segurança. Vista bata, luvas de látex descartáveis e óculos protectores sempre que necessário.
6. Reagentes do kit contendo material perigoso podem causar irritação da pele e dos olhos. Veja MATERIAIS FORNECIDOS e os rótulos para mais detalhes. As Fichas de Segurança do produto para este kit estão disponíveis na Homepage da IBL ou a pedido directamente à IBL:
7. Químicos e reagentes preparados ou utilizados devem ser tratados como resíduos perigosos de acordo com as normas nacionais de segurança e resíduos perigosos.
8. Alguns reagentes contêm azida de sódio (NaN3) como conservante. Em caso de contacto com os olhos ou pele, enxagúe imediatamente com água. A NaN3 pode reagir com o chumbo e o cobre da canalização para formar azidas metálicas explosivas. Quando descartar os reagentes, enxagúe com grande volume de água para evitar a formação dos compostos.
9. Todos os componentes deste kit contendo soro ou plasma humano foram testados e foram considerados não reactivos para HIV I/II, AgHBs e HCV. No entanto, não é possível excluir em absoluto

a presença destes ou outros agentes infecciosos e portanto os reagentes devem ser tratados com potencialmente perigosos quer na sua utilização quer na sua eliminação.

5. ARMAZENAMENTO E ESTABILIDADE

O kit é enviado à temperatura ambiente e deve ser armazenado de 2 a 8ºC. Mantenha-o longe do calor ou da luz solar directa. A estabilidade e armazenamento das amostras e reagentes preparados são referidos nas secções correspondentes.
A microplaca é estável até expirar a data de validade do kit, na embalagem aberta mas firmemente fechada, quando armazenada a 2-8℃.

6. RECOLHA E ARMAZENAMENTO DE AMOSTRAS

Saliva

O paciente não deve comer, beber, mastigar pastilha elástica ou lavar os dentes nos 30 min anteriores à obtenção da amostra. Caso contrário, deve lavar vigorosamente a boca com água fria 5 min antes da recolha da amostra. Não recolher amostras se houver doenças, inflamação ou lesões orais (contaminação por sangue).

A saliva pode ser recolhida com equipamento de recolha apropriado. Deve ser recolhido no mínimo 0.5 mL de líquido. O fluxo de saliva pode ser estimulado mastigando um pedaço de Parafilm®. Recomenda-se o congelamento das amostras a –20℃ antes dos testes laboratoriais. Após descongelar, mistura e centrifugar a 2000 – 3000 x g para remover quaisquer partículas.

⚠ **Assegurar que as amostras de saliva estão com bom aspecto (sem cor avermelhada indicando contaminação por sangue).**

Armazenamento:	37℃	18-25℃	2-8℃	≤ -20℃ (Alíquotas)
Estabilidade:	1 sem	> 2 sem	> 4 sem	≥ 6 meses

Soro, Plasma (EDTA)

Devem ser observados os cuidados usuais para a punção venosa. É importante preservar a integridade química da amostra de sangue desde o momento da colheita até ao momento de ser analisada. Não utilize amostras muito hemolíticas, ictéricas ou lipémicas. Amostras que apresentem turvação deverão ser centrifugadas antes do teste e devem ser removidas as partículas de matéria.

Armazenamento:	2-8℃	≤ -20℃ (Alíquotas)	Manter afastado do calor ou luz solar directa. Evitar congelar-descongelar repetidamente.
Estabilidade:	48 h	6 meses	

7. MATERIAIS FORNECIDOS

Quantidade	Símbolo	Componente
1 x 12x8	MTP	**Microplaca** Tiras separáveis. Revestida com antisoro anti-cortisol (coelho).
1 x 10 mL	ENZCONJ	**Conjugado Enzimático** Colorido amarelo. Pronto a usar. Contêm: Cortisol (purificado cromatograficamente), conjugado com HRP, estabilizantes.
1 x 2.5 mL 6 x 0.5 mL	CAL A-G	**Padrão A-G** 0; 0.03; 0.06; 0.20; 0.60; 1.50; 4.00 µg/dL 0; 0.3; 0.6; 2.0; 6.0; 15; 40 ng/mL 0; 0.83; 1.7; 5.5; 17; 41; 110 nmol/L Pronto a usar. Contêm: Cortisol, Tampão, 0.1 % BSA, 0.1 % ProClin.
2 x 0.5 mL	CONTROL 1+2	**Controlo 1+2** Pronto a usar. Contêm: Cortisol, baixo e elevado, Tampão, 0.1 % BSA, 0.1 % ProClin. Para concentrações exactas consultar etiquetas dos frascos.
1 x 12 mL	TMB SUBS	**Solução de Substrato TMB** Pronto a usar. Contêm: TMB, Tampão, estabilizantes.
1 x 12 mL	TMB STOP	**Solução de Paragem TMB** Pronto a usar. 1 M H_2SO_4.
1 x 50 mL	WASHBUF CONC	**Tampão de Lavagem** Concentrado (10x) Contêm: tampão fosfato, Tween, estabilizantes.
3 x	FOIL	**Película Aderente**

8. MATERIAIS NECESSÁRIOS MAS NÃO FORNECIDOS

1. Pipetas (Multipette Eppendorf ou aparelhos semelhantes, < 3% CV). Volumes: 5; 20; 50; 100; 1000 µL
2. Deve ser usado um dispositivo de amostragem adequado. (pode ser encomendado separadamente à IBL pela REF RE69991)
3. Padrão zero adicional para diluição do soro (Pode encomendar-se separadamente à IBL com a REF KECO611)
4. Controlos de Soro (ex: "Lyphochek Immunoassay Plus Control", Biorad, Alemanha)
5. Agitador orbital (400-600 rpm)
6. Vortex
7. Pipeta de 8 canais com reservatório de reagente
8. Recipiente de lavagem, sistema de lavagem de microplacas automático ou semi-automático
9. Leitor de microplacas com capacidade de ler absorvâncias a 450 nm (comprimento de onda de referência 600-650 nm)
10. Água bidestilada ou bi-destilada
11. Toalhas de papel, pontas para pipetas e cronómetro

9. NOTAS SOBRE O PROCEDIMENTO

1. O manuseamento incorrecto da amostra ou alterações no procedimento do teste podem influenciar os resultados. Os volumes de pipetagem indicados bem como os tempos de incubação, a temperatura e os passos de pré-tratamento devem ser realizados estritamente de acordo com as instruções. Utilize apenas pipetas e instrumentos calibrados.
2. Uma vez iniciado o teste, todos os passos devem ser executados sem interrupção. Garanta que os reagentes necessários, os materiais e dispositivos são preparados e prontos a usar no tempo apropriado. Todos os reagentes e amostras devem estar à temperatura ambiente antes de utilizar (18 - 25ºC). Agite cuidadosamente todos os frascos de reagentes líquidos e a amostra antes de utilizar. Agite os reagentes sem formar espuma.
3. Evite a contaminação dos reagentes, pipetas e poços/tubos. Utilize pontas novas descartáveis para cada reagente, padrão ou amostra. Não troque as tampas. Feche sempre os frascos não utilizados. Não reutilize poços/tubos ou reagentes.
4. Recomenda-se a determinação em duplicado das amostras de maneira a identificar potenciais erros de pipetagem.
5. Utilize um esquema de pipetagem para verificar uma disposição apropriada na placa.
6. O tempo de incubação afecta os resultados. Todos os poços devem ser manuseados pela mesma ordem e na mesma sequência de tempo. E recomendável utilizar uma Micropipeta de 8 canais para a pipetagem das soluções nos poços.
7. A lavagem da microplaca é importante. Poços mal lavados originam resultados errados. É recomendável utilizar uma pipeta multicanal ou um sistema automático de lavagem de microplacas. Não permitia que os poços sequem entre lavagens. Não arranhe as paredes dos poços durante a lavagem e aspiração. Encha todos os reagentes com cuidado. Enquanto lava, verifique que todos os poços são cheios com o Tampão de Lavagem e que não há resíduos nos poços.
8. A humidade afecta os tubos/poços revestidos. Não abra o saco até atingir a temperatura ambiente. Os tubos/poços não utilizados devem ser automaticamente guardados no saco incluindo o dessecante.

10. INSTRUÇÕES PRÉ-TESTE

10.1. Preparação de Componentes liofilizados ou concentrados

Diluir/ dissolver	Componente		Diluente	Relação	Observações	Armazenamento	Estabilidade
10 mL	**Tampão de Lavagem**	juntar 100 mL	agua bidest.	1:10	Misturar energicamente.	2-8℃	4 sem

10.2. Diluição de Amostras

Amostra	diluir	com	Relação	Observações
Saliva	não	-	-	-
Soro	geralmente	Padrão A	1:50	p.ex. 5 µL + 245 µL

As amostras que contenham concentrações superiores à do padrão mais elevado têm que ser novamente diluídas até 1:32 com Padrão A e re-analisadas.

11. PROCEDIMENTO DO ENSAIO

1.	Pipetar **50 µL** de cada **Padrão**, **Controlo** e **amostra** para os respectivos poços da microplaca.
2.	Pipetar **100 µL** de **Conjugado Enzimático** para cada poço. Tapar a placa com nova película aderente.
3.	**Incubar 2 h** à **TA (18-25°C)** num agitador orbital (400-600 rpm).
4.	Remover a película adesiva. Rejeitar a solução de incubação. Lavar a placa **4 x** com **250 µL** de **Tampão de Lavagem** diluído. Remover o excesso de solução batendo com a placa invertida numa toalha de papel.
5.	Pipetar **100 µL** de **Solução de Substrato TMB** para cada poço.
6.	**Incubar 30 min à TA (18-25°C)** num agitador orbital (400-600 rpm).
7.	Parar a reacção de substrato adicionando **100 µL** de **Solução Stop TMB** a cada poço. Misturar rapidamente os conteúdos, agitando cuidadosamente a placa. A cor muda de azul para amarelo.
8.	**Medir** a densidade óptica com um fotómetro a **450 nm** (Comprimento de onda de referência: 600-650 nm) nos **15 min** seguintes à pipetagem da Solução Stop.

12. CONTROLO DE QUALIDADE

Os resultados do teste só são válidos se o teste for executado de acordo com as instruções. Adicionalmente o utilizador deve cumprir rigorosamente as Boas Práticas de Laboratório ou outras leis ou regulamentos. Todos os padrões devem estar dentro dos intervalos de aceitação definidos no Certificado de CQ. Se não cumprirem os critérios a série não é válida e deve ser repetida.
Cada laboratório deve usar amostras conhecidas como controlos adicionais.
Em caso de desvios os seguintes aspectos técnicos devem ser tidos em conta: datas de validade dos reagentes (preparados), condições de armazenamento, pipetas, dispositivos, condições de incubação e métodos de lavagem. É recomendável participar em ensaios de garantia da qualidade apropriados.

13. CÁLCULO DE RESULTADOS

Constrói-se um gráfico com a DO dos padrões (eixo dos yy, linear) em função da sua concentração (eixo dos xx, logarítmico), quer em papel gráfico semi-logarítmico quer usando um método computorizado. Uma boa curva é fornecida optando por interpolação ”cubic spline”, ajustamento 4PL (4 parameter logistics) ou modelo “logit-log”.

Para o cálculo da curva de calibração, deve usar cada sinal dos padrões (se um dos duplicados apresentar um valor claramente diferente do esperado pode ser omitido e o valor mais razoável pode ser usado).

A concentração das amostras pode ser lida directamente a partir da curva de calibração.
Devido à diluição das amostras de soro, os valores obtidos para soro têm que ser multiplicados pelo factor 50.

Amostras que apresentem concentrações acima do padrão mais elevado, têm que ser diluídas como descrito em INSTRUÇÕES PRÉ-TESTE e testadas novamente.

No caso de amostras diluídas os valores têm que ser multiplicados pelo factor de diluição correspondente.

Amostras de saliva com valores consideravelmente elevados devem ser novamente verificadas relativamente a contaminações com sangue.

Conversão:

Cortisol (ng/mL) x 2.76 = nmol/L

Cortisol (µg/dL) x 27.6 = nmol/L

Reportable range:

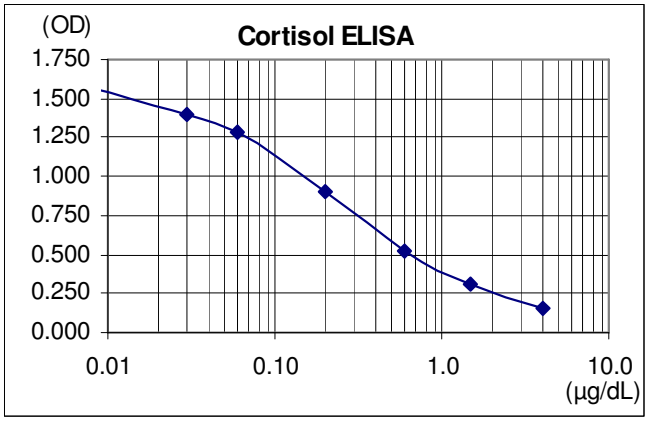
Saliva: 0.015 – 4 µg/dL Cortisol

Serum: 0.75 – 200 µg/dL Cortisol

Curva de Calibração Típica

(Exemplo. Não usar para cálculos!)

Padrão	Cortisol (µg/dL)	$OD_{Média}$	OD/OD_{max} (%)
A	0.00	1.699	100
B	0.03	1.401	82
C	0.06	1.278	75
D	0.20	0.905	53
E	0.60	0.524	31
F	1.50	0.304	18
G	4.00	0.155	9



Standards and Controls are calibrated by use of an isotope dilution-GCMS as reference method (Siekmann et al., J Clin Chem Clin Biochem 1982;20:883-892).

14. VALORES ESPERADOS

Os resultados por si só não devem ser a única razão para qualquer acção terapêutica e deverão ser correlacionados com outras observações clínicas e testes de diagnóstico.

Indivíduos aparentemente saudáveis apresentam os seguintes valores:

	n	µg/dL		nmol/L	
		manhã	tarde	manhã	tarde
Saliva	340	0.20 – 1.41	0.04 – 0.41	5.52 – 28.92	1.10 – 11.32
Soro	125	5 – 25	2 – 12	138 - 690	55.2 – 331.2

Tempo após acordar (h)	Cortisol (Saliva) Limite (♂/♀; > 6 anos; n = 110; 5% - 95% percentil)			
	Mediana (nmol/L)	Limite (nmol/L)	Mediana (µg/dL)	Limite (µg/dL)
0 – 1.5	18.9	5.1 - 40.2	0.685	0.185 – 1.457
1.5 – 3.0	11.8	3.6 - 28.4	0.428	0.130 – 1.029
3.0 – 6.0	6.7	2.1 - 15.7	0.243	0.076 – 0.569
6.0 – 9.0	5.5	1.8 - 12.1	0.199	0.065 – 0.438
9.0 – 15.0	3.3	0.9 - 9.2	0.120	0.033 – 0.333

(Westermann J, Demir A, Herbst V. Determination of Cortisol in Saliva and Serum by a Luminescence-Enhanced Enzyme Immunoassay. Clin Lab 2004;50:11-24)

Recomenda-se que cada laboratório estabeleça o seu próprio intervalo de normalidade.

15. LIMITAÇÕES DO PROCEDIMENTO

Os níveis de crianças ainda não foram avaliados com este teste.

A recolha de amostras tem um efeito significativo nos resultados dos testes. Ver RECOLHA E ARMAZENAMENTO DE AMOSTRAS para detalhes.

Para reacções cruzadas, ver DESEMPENHO.

Nota: As amostras contendo timerosal não devem ser usadas no ensaio.

Os seguintes componentes sanguíneos não têm um efeito significativo (+/- 20% do esperado nos resultados do teste até às concentrações descritas em baixo:

	Saliva	
	Conc.	**Cortisol (µg/dL)**
Sangue	0.25 %	0.16; 0.26; 1.09
NaN_3	0.25 %	0.18; 0.21; 0.33
	Soro	
	Conc.	**Cortisol (µg/dL)**
Hemoglobina	1.0 mg/mL	20.4; 15.8
Bilirrubina	1.0 mg/mL	20.4; 15.8
Triglicéridos	50 mg/mL	20.4; 15.8

16. DESEMPENHO

Especificidade Analítica (Reacção Cruzada)	Substância		Reacção Cruzada (%)	Reactividade cruzada de outras substâncias testadas < 0.01 %
	Prednisolona		30	
	11-Desoxi-Cortisol		7.0	
	Corticosterona		1.4	
	Cortisona		4.2	
	Prednisona		2.5	
	17α-OH-Progesterona		0.4	
	Desoxi-Corticosterona		0.9	
	6α-Metil-17α-OH-Progesterona		0.04	
Sensibilidade Analítica (Limite de Detecção)	0.005 µg/dL	Média do Sinal (Padrão Zero) - 2SD		
Sensibilidade Funcional	0.030 µg/dL	Média Conc. < 20 % CV		

Precisão	Saliva (n = 20)			Soro (n = 20)		
	Conc. (µg/dL)	SD (µg/dL)	CV (%)	Conc. (µg/dL)	SD (µg/dL)	CV (%)
Intra-Ensaio	0.27	0.019	7.3	1.8	0.174	9.9
	1.48	0.063	4.2	11.5	0.766	6.7
	2.34	0.073	3.1	26.2	1.554	5.9
Inter-Ensaio	0.54	0.047	8.8	1.5	0.305	20
	1.29	0.120	9.3	11.6	2.006	18
	2.35	0.151	6.4	20.3	2.712	13

Linearidade	Saliva			Soro				
	Diluição	Medido (µg/dL)	Rec. (%)	Diluição	Medido (µg/dL)	Rec. (%)		
	-	0.67	100	1:50	59.2	100		
	1:2	0.29	85	1:100	30.8	104		
	1:4	0.16	93	1:200	16.1	109		
	1:8	0.09	104	1:400	8.6	116		
	1:16	0.04	93	1:800	4.3	116		
	1:32	0.02	115	1:1600	1.7	91		
	-	0.33	100	1:50	59.3	100		
	1:2	0.17	104	1:100	29.4	99		
	1:4	0.08	99	1:200	16.4	111		
	1:8	0.04	98	1:400	7.5	101		
	1:16	0.02	107	1:800	4.3	117		
	1:32	0.01	117	1:50	1.7	94		
	-	1.65	100	1:100	70.9	100		
	1:2	0.83	100	1:200	35.7	101		
	1:4	0.41	102	1:400	18.5	104		
	1:8	0.21	113	1:800	10.2	115		
	1:16	0.10	107	1:1600	5.0	113		
	1:32	0.05	109	1:3200	2.2	99		
Recuperação	Saliva				Soro			
	Conc. (µg/dL)	Adicionado (µg/dL)	Medido (µg/dL)	Rec. (%)	Conc. (µg/dL)	Adicionado (µg/dL)	Medido (µg/dL)	Rec. (%)
	Saliva 1 (0.13)	-	0.13	100	Soro 1 (10.9)	-	10.9	100
		0.02	0.16	106		1.5	11.8	95
		0.06	0.17	87		3.1	13.8	99
		0.20	0.28	86		10.2	19.4	92
		0.60	0.61	83		30.6	38.4	92
		1.50	1.37	83		76.5	79.9	91
	Saliva 2 (0.16)	-	0.16	100	Soro 2 (14.0)	-	14.0	100
		0.02	0.17	97		1.3	14.3	94
		0.06	0.20	92		2.6	14.9	90
		0.20	0.30	84		8.7	20.5	90
		0.60	0.66	87		26.0	33.8	85
		1.50	1.53	92		65.0	77.3	98
						173.4	191.2	102
	Saliva 3 (0.15)	-	0.15	100	Soro 3 (19.5)	-	19.5	100
		0.02	0.16	91		1.5	21.4	102
		0.06	0.22	102		3.1	23.9	106
		0.20	0.33	94		10.2	28.6	96
		0.60	0.74	98		30.6	49.9	100
		1.50	1.54	88		76.5	93.0	97
Comparação do método	Saliva	IBL-ELISA = 1.09 x IBL-Imunoensaio de luminescência + 0.01					r = 0.996; n = 82	
	Soro	IBL-ELISA = 1.06 x IBL-Imunoensaio de luminescência - 1.44					r = 0.987; n = 60	
		IBL-ELISA = 0.84 x GCMS + 0.36					r = 0.918; n = 33	

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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