UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE FARMACIA

Departamento de Farmacología

TESIS DOCTORAL

Salivary Biomarkers of Stress in Psychological Patients on Fluoxetine Therapy

Biomarcadores Salivales de Estrés en Pacientes Psicológicos en Tratamiento con Fluoxetina

MEMORIA PARA OPTRA AL GRADO DE DOCTOR PRESENTADA POR

Nuha Mohammed Yahya Al-Dallee

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CERTIFICAN:

Que la presente Tesis Doctoral presentada por Dña. **Nuha Mohammed Yahya AL-Dallee,** Titulada "Salivary Biomarkers of Stress in Psychological Patients on Fluoxetine Therapy", ha sido realizada bajo nuestra inmediata dirección y asesoramiento.

Concluido el trabajo experimental y bibliográfico, autorizamos su presentación para sea juzgada por el Tribunal correspondiente.

Madrid, Abril 2017

Dra. María Teresa Ortega Hernández-Agero

Dra. Nahla O.M. Tawfiq

DEDICATION

TO MY BELOVED COUNTRY IRAQ.

TO THE PEOPLE OF MOSUL CITY, MOSUL WILL RETURN BETTER.

TO MY FATHER & MOTHER.

*** TO MY LOVELY HUSBEND AND MY GREAT LOVE (BAZIL). TO OUR LIFE AND FUTURE "WASEEM"***

TO MY SISTERS & BROTHERS.

TO MY FRIENDS.

TO ALL MEDICAL STUDENTS.

WITH MY BEST WISHES
AND
RESPECT TO ALL

NUHA

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Nuha M.Y. Al-Dallee

LIST OF ABBREVIATIONS

5-HT	5-hydroxy tryptamine
AA	Alpha amylase enzyme
CNP	Chloronitrophenol
d	Day
DSM-I	Diagnostic and Statistical Manual of Mental Disorders-First Edition
DSM-II	Diagnostic and Statistical Manual of Mental Disorders- Second Edition
DSM-III	Diagnostic and Statistical Manual of Mental Disorders- Third Edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders- Forth Edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders- Fifth Edition
ELISA	Enzyme-Linked Immuno-Sorbent Assay
EPS	Extrapyramidal syndrome
F	Female
FDA	United States Food and Drug Administration's
FR	Flow rate
GAD	Generalized anxiety disorder
HPA axis	Hypothalamus-pituitary-adrenal axis
HPLC	High Performance Liquid Chromatography
ICD	International Classification for Diseases and Related Disorders
IgA	Immunoglobulin A
ISE	Ion selective electrode reader
K+	Potassium ion
L	Liter
M	Male
MAOIs	Monoaminooxidase inhibitors
MDD	Major Depressive Disorder
mg	Milli gram
min	Minute
ml	Milli liter
mmol	Milli mole

Na^+	Sodium ion
NDRIs	Norepinephrine and Dopamine Reuptake Inhibitors
ng	Nanogram
NS	Not Significant
OCD	Obsessive compulsive disorders
OD	Optical density
PMDD	Pre Menstrual Dysphoric Disorder
PTSD	Post-traumatic stress disorder
rpm	Rounds per minute
SAA	Salivary alpha amylase
SAM	Sympathetic-adrenal- medullary
SD	Standard deviation
SERT	Serotonin Transporter
SF	Salivary flow
SIADH	Syndrome of inappropriate anti diuretic hormone
S-IgA	Secretary IgA
SNRIs	Serotonin and Norepinephrine Reuptake Inhibitors
SSRIs	Selective serotonin reuptake inhibitors
$t_{1/2}$	Half life
TCAs	Tricyclic antidepressants
U	Unit
VAS	Visual analogue scale
WHO	World Health Organization
XQ	Xerostomia questionnaire

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Abstract

In the last recent decades, stress has become an inevitable part plaguing the daily lives. Psychological stress has a negative impact over physical, mental and social well-being of a person. It has been suggested to play an important role in causation or precipitation of multitude of medical and dental problems ranging from serious heart diseases, cancers, gastrointestinal diseases, to common headaches, migraine, recurrent oral ulcerations, burning and dry mouth.

In order to better understand the role of stress, valid and reliable measurement of stress is of utmost importance. Since, most of methods used for the diagnosis of stress are questionnaires form (subjective methods) which depend on person and there is many inter individual variation between subjects, thus there is increased need of biological stress markers to provide an objective, reliable and authentic evidence of stress. Self-reporting subjective questionnaire forms in stress evaluation and as psychological diagnostic mean provide highly inconsistent results according to patient's mood and attitude. And this is because many individuals suffering from stress related problems have a tendency to either negate or exaggerate the real condition; which may lead to a deviation in the study and discompose the results. Thus there is a high need for studying the changes caused by stress in the human body to evaluate the usefulness of the bio physiological indicators or biomarkers to provide a reliable objective evaluation and assessment of a stress and psychologically related conditions. Salivary biomarkers are one of most widely researched area of interest because it might to provide a reliable, noninvasive and objective measurements of the response of the body, also because of easy and rapid collection of samples compared to the blood and urine samples thereby increasing the patient compliance. Many biomarkers have been used in determination of stress such as cortisol levels, immunoglobulins, cardiovascular parameters. Studies showed there is an evidence of sensitivity of SAA levels in response to different stressful conditions, physically and psychologically.

Changes in SAA is thought to have implications for health. And consider a promising role of salivary alpha amylase as a possible biological stress marker. The release of salivary alpha amylase enzyme was reported to react to physiological and psychological stressors thus it might be a biomarker of stress which consider very important and valuable biological markers in psychophysiological research and clinical practice.

Abstract

It’s very difficult to describe the situation in IRAQ and there is no accurate statistics in all fields especially in health sector which has been affected thoroughly by the war and the number of subjects need psychological health care has been increased due to the increased stress, violence due to the post war conflicts. For these reasons its necessary to find simple and economical method to detect subjects under stress to help in the prevention of deterioration of their mental state and progress to a more serious condition. This will be the prime objective of this study.

In this study, in view of the shortage of data in IRAQ especially from 2003 and with increased susceptible of people to violence and stress and increase susceptibility to psychiatric disorders and increase need for drug treatments. This study was to record type of psychiatric disorders in sample of different patients as an example that may reflect the population, adverse effects of most widely used fluoxetine therapy in our population as a goal to reach maintenance of optimal functioning and well-being and as try to achieve “keep people healthy” as opposed to “how well it cures diseases and the consequence of adverse drug therapy”, as a part, encourage the patients to concern with oral hygiene and maintain their oral health as a part of the general health and to report any dental problems including adverse effects of drugs to the psychiatrist.

Drug use is associated with significant detrimental, psychological, nutritional, and social changes, any of which can affect the general and oral health. Fluoxetine was the first SSRIs approved to treat depression in humans and is one of the most widely prescribed antidepressant drugs. Many general and oral adverse effects have been reported with fluoxetine therapy. Some of its observed secondary effects are related to an alteration in the salivary secretion and composition. Despite that fluoxetine was approved by the United States Food and Drug Administration's (FDA) in 1987 for the treatment of depression and then for the treatment of a number of psychiatric disorders in adults and children, it was used in the psychiatric treatment in IRAQ in the ultimate years and there is shortage of data that record efficacy, adverse effects and other about drug due to the non-recognition of the psychiatric disorders and mainly depression by the majority of the society. Most of the people in middle east does not accept the concept of (pharmacological treatment) for the psychiatric disorders especially depression because of some religious believes and social aspects. This study was the first pharmacological study done in Mosul and Iraq on the psychological patients to see the effects of the drug (fluoxetine) on the patients and encourage both the psychiatrist and the patients to detects the adverse effects of the drug and try to avoid it as possible.

Abstract

Saliva is one of the most important factors in regulating oral health, with flow rate and composition changing throughout development and during disease and treatment with different type of medications like SSRIs. Fluoxetine is one of the class termed SSRIs which is a serotonin agonist. Many general and oral adverse effects have been reported with fluoxetine therapy.

The aims of the present study were to measure the effects of psychiatric diseases and their treatment with SSRIs (fluoxetine) on the salivary flow rate and contents (salivary alpha amylase, sodium, and potassium). Also, to evaluate the adverse effects (general and orofacial) of fluoxetine therapy and its relationship with the dose and duration of the treatment.

The specific aims of the study were to investigate:

1. The usefulness of salivary alpha amylase (SAA) as a good biological marker of stress in psychological patients and check the possibility to use it to detect person on stress like a psychological patient.
2. Salivary sodium and potassium, as markers of stress and check the possibility to use them to detect person on stress in conjunction to SAA to detect patients.
3. The biochemical changes in saliva including sodium, potassium and salivary alpha amylase concentrations, and changes in salivary flow rate associated with fluoxetine therapy in psychiatric patients.
4. The effects of psychiatric diseases on the composition of saliva (salivary alpha amylase, sodium and potassium) and salivary flow rate.
5. Objective and subjective measurement of xerostomia and determination of its grades as a result of disease process and fluoxetine therapy.
6. The adverse effects associated with fluoxetine therapy in our population including both general adverse effects on the body at whole and oral adverse effects and their relation with dose and duration of therapy.
7. Fluoxetine concentrations in serum by high performance liquid chromatography (HPLC) and their relation to SAA, Na^+, K^+ concentrations and adverse effects of fluoxetine administration.

Materials and Methods: The study including eighty individuals divided in to two major groups of study:

1. **The study group (Patients)**: consisted of (60) patients with age range was between (15-56) years which divided into two subgroups to investigate the effects of fluoxetine therapy in the patients in short (acute) and long durations (chronic) and as follows:

   1.1. The Acute group consisted of (39) patients with different psychological diseases (23 males, 16 females) which have been provided with fluoxetine capsules and starting fluoxetine therapy after two weeks wash out period and have been followed for (2 months). Their average age was (35.4±10.7) years.

   1.2. The Chronic group 21 patients with different psychological diseases (7 males, 14 females); their average age was (33.95±10.7) years. These patients were already on fluoxetine therapy for different doses and different durations.

2. **The Control group**: included twenty nonsmoker healthy subjects (10 males, 10 females) their average age (35.6±13.9) range (17–59years).

Saliva and blood samples and standard questionnaire form including visual analogue scale (VAS) were obtained from each individuals in each visit (one visit for chronic group and three for acute group (pre, post 4weeks and post 8weeks of treatments). Salivary (alpha amylase, sodium ion, potassium ion, fluoxetine concentration by HPLC and flow rate) were analyzed. Also the symptoms and adverse effects of fluoxetine reported in the clinical reports of the patients have been recorded and analyzed in each visit.

Results and Discussion:

Salivary alpha amylase activity in patients showed statistically significant higher values when compared to control healthy group at ($p=0.0001$). Also when comparing the SAA activity of the patients with the healthy levels, we notice that SAA had the ability to distinguish 76.9% of the patients at pretreated level and 85.7% of the patients in the chronic group and this percent was increased by using the SAA, Na^+ and K^+ concentration measurements together to detect psychological patients which can be considered as a good high percent of detection and might indicate that they are a promising good biomarkers of stress in psychological conditions.

Depending on the physiological response to stress, we can explain the increment in the SAA activity. Psychosocial stress is known to induce various responses of physiologic systems with particular increasing activities in the hypothalamus-pituitary-

adrenal axis (HPA) reflected by cortisol secretion as well as in the sympathetic-adrenal-medullary (SAM) system reflected by salivary alpha amylase level. Many studies study the salivary activity after acute stressor physical or psychological such as (Granger *et al*., 2006; Nater *et al.*, 2006 Malamud and Rodriguez-Chavez, 2011). Granger *et al*. in the study done in 2007 said "An increase in SNS activity leads to higher levels of alpha-amylase production, which can be measured by examining saliva samples ". Multiple studies have examined the relationship between norepinephrine and SAA because both are associated with SNS activation. However, the results have been inconsistent such as (Chatterton *et al*., 1996; Nater *et al*., 2006).

Allwood *et al*., (2011) found results suggesting that SAA is more reactive to laboratory stressors (performance or peer rejection tasks) than cortisol. This is the first study that examines the SAA activity in acute and chronic state and in psychological patients with different psychological situations and compare it with other salivary electrolytes and with the adverse effects reported in the patients. Our sample size was less to represent the general population, but it gives an idea about the salivary changes that accompanying the stress and psychological conditions and thus more studies was needed with large numbers to confirm our findings.

The salivary changes reported in groups in this study were as follows:

In the disease condition comparing with the healthy control, this study showed significant higher levels of SAA and Na^+, while significant lower K^+ level in the disease condition than in the healthy control at $p<0.05$. Also reported significant lower FR and significant higher VAS than the healthy control at $p<0.05$. Significant nervousness, headache, insomnia, change appetite, xerostomia, and dysgeusia than the healthy control at $p<0.05$ have been reported in this study.

Release of SAA is regulated by autonomic innervations (Bagan-Sabastain, 2004) and usually higher level of SAA produced by increased sympathetic activity (Granger *et al*., 2006; 2007). SAA activity increased as a normal response of the body to stress and this mediated by sympathetic adreno medullary system, in which SAA level considered as a good biomarker to its activity (Friedlander and Mahler, 2001). While significant higher Na+ and significant lower K+ level reported in the disease condition than in the healthy control at $p<0.05$ this may be due to that most of the patients in this study were depressed and even suffer from other psychiatric disorders might have been associated with depression. The explanation would depend on this to explain our finding. Depression known to be associated with increased level of aldosterone which lead to increase Na^+ re

absorption and exchange of K^+ this will explain the increased level of Na^+ and decreased level of K^+ in the disease condition when compare with healthy individuals (Zanatta *et al.*,2001).

In the study group (chronic patients), the SAA level showed significant decrease with fluoxetine therapy at $p<0.05$. While, Na^+ and K^+ reported significant differences with the disease pre level. The concentration of Na^+ was low, while K^+ was high than in the disease condition. Significant changes in the salivary flow rate and VAS between the chronic group and the pretreated level.

In the study group (acute or follow up patients for two month of fluoxetine therapy), significant differences have been reported between different readings (pre, post1, post2) of the acute group in the SAA output**.** Although, the level showed decliner with the treatment, but it still higher than the healthy level. In this study, it was found that the SAA output was significantly higher in the disease condition (represented by acute and the chronic group in this study) compared with the healthy control condition. Significant differences were found between the base line pretreated level (19.87u/min) which was higher than the treated post1 (12.86 u/min) and post2 (11.43 u/min) measures which indicated the efficacy of the treatment to reduce psychological stress associated with the psychological conditions. This is in agreement with (Noto *et al.*, 2005) were they found that salivary alpha amylase is a useful indicator of psychological stress. The changes in SAA levels may be related to the activation of the β-adrenergic system and reflects the psychological stress in depression and other psychiatric disorders. However, the results of Kivlighan and Granger (2006) and Inagaki *et al*. (2010) which indicated a predominant role of the sympathetic nervous system in the secretion of SAA together with parasympathetic withdrawal, under psychosocial stress, support our suggestion that psychological factors (like stress) effects SAA secretions and concentrations. Thus, as a result to body response to fluoxetine therapy and efficacy in treating depression and psychological disorders and improved in mood due to the increased 5-HT level and reduce the effect of stress on the patients. This will have appeared in the decliner in SAA secretion which reflect the decrease in sympathetic activity.

Na^+ reported significant changes between pre and post treated level in the acute group, while K^+ showed no significant changes. The pattern of changes was decrement in Na^+ and increment in K^+ with fluoxetine therapy. The pretreated level was significantly higher than the post treatment level with fluoxetine at $p<0.05$. Published studies do not offer any explanations for dramatic changes in K^+ and Na^+ concentration in the psychiatric

patients on fluoxetine therapy. It is interesting that many of patients in this study showed salivary changes such as significant changes reported between the disease condition (patients chronic and acute groups which was on fluoxetine therapy) when compared with the healthy control subjects. Based on published studies on non-psychiatric patients, it is attempted to supply some explanations. As the salivary flow rate increases, the concentrations of total protein, Na^+, calcium, chloride, and bicarbonate, as well as the pH increases to various levels (Edgar, 1992; Tenovuo *et al*., 1994) which may explain these findings that the Na^+ showed a pattern of decliner with the treatment and it reached to the healthy control level in the post 2 level. This may be as a result of the decreased in salivary flow that have been reported with treatment and this was also in agreement with (Höld *et al*., 1999) which propose direct relation of Na^+ secretion with the salivary flow rate. This also may explain the pattern of increment with treatment that have been reported with fluoxetine therapy which may be due to the indirect relation of K^+ secretion with FR as mentioned by Höld *et al*., (1999). Another explanation to the decrease and increase in Na^+ and K^+ conc. respectively was that the composition of saliva is subject to hormonal modulation (Wotman *et al.,* 1973; Kakmoto *et al.*, 1988) which act directly to increase Na^+ reabsortion and K^+ secretion. Also this supported by the finding of (Zanatta *et al*., 2001) which found that fluoxetine was increase the Na-K ATPase activity by 27% than the control which might lead to increase Na^+ re absorption and K^+ excretion.

According to all of the above, and by comparison of the concentrations of Na^+ and K^+ with the concentrations mentioned in other studies we can notice the higher concentrations of these parameters in our sample compared with others, which may be a serious or even dangerous indicator to the severe and continuous stress that our population live in. This exciting finding may give rise to many studies that should be done to investigate the effect of such acute and continuous stress on the performance and wellbeing in our population and its effect on cognitive functions and memory. Also, on the ability to make a decision. According to this point of view, that needs more studies to measure the effect of acute and chronic stress and anxiety in our population, not only to study it, but to try to find some solutions or methods to compensate or deal with it.

Assessment of safety and tolerability showed that 16 patients (41.02%) out of 39 patients were withdrawn from our study, 8 patients (20.51%) due to the adverse effects (G.I.T, insomnia, sexual adverse effects, and TMJ problems) and 8 (20.51%) due to lack of efficacy and non-complaint. The main adverse effects caused by fluoxetine therapy were breathing difficulties, abnormal dreams, drowsiness, insomnia, headache, weight

loss, change in appetite, tremor, myalgia, anorexia, nausea, diarrhea, anxiety, taste changes (dysgeusia), xerostomia, and TMJ problems.

The effect of gender on parameters showed that from all salivary parameters: Only Na^+ concentration was significantly high in females than males in the chronic group. While general adverse effects including (anxiety, headache, nervousness) were significantly higher in males than females in the chronic group while anxiety, constipation, palpitation were significantly higher in males than females in the acute group. Only dysgeusia was significantly high in males than females in the chronic group while TMJ problems was significantly high in males than females in the acute group.

In relation to dose, Na^+ and nervousness showed a significant increase level with dose 40 mg/d of fluoxetine in the chronic group at ($p=0.04$ and $p=0.034$ respectively), and only anxiety in the acute group showed significant higher incidence with dose 60mg/d at $p=0.035$.

The FR decreased with the duration while the VAS showed high score with long term treatment. Weight loss, myalgia, constipation, palpitation, breathing difficulties, nervousness, anxiety, insomnia, nausea, taste changes, xerostomia, TMJ problems and black hairy tongue showed significant increase with the increased duration of therapy at $p<0.05$.

All patients being treated with antidepressants for any indication should be monitored appropriately and observed closely for clinical worsening, suicidality, and unusual changes in behavior, especially during the initial few months of a course of drug therapy, or at times of dose changes, either increases or decreases. And for that purpose HPLC is the most commonly used mean for quantitative serum measurements of a medications. Many factors might influence the measured plasma drug concentrations, including variations in absorption, distribution, and metabolic clearance rates that are influenced by genetic and other differences among individuals. Determination of the relationship between fluoxetine concentrations and clinical response remains a questionable and also the relations between fluoxetine conc. and the adverse effects reported by patients.

In this study, a wide range of fluoxetine concentration has been reported in relation to dose. All was within the reported therapeutic levels.

In general, the mean concentration reported in this study was higher in females than males in the chronic and acute groups (Ferguson, 2006; Blazquez *et al.*, 2014). Amsterdam *et al*. had found in his study in (1997) that males had lower fluoxetine and norfluoxetine

serum levels while Pato *et al.* in his study (1991) said that sex and age of the patient did not impact metabolism and drug concentrations of fluoxetine. Also in this study, no clear relation noticed between drug concentration and a patients' withdrawal from the study but these patients reported a higher range of fluoxetine concentration in the previous reading comparing with other patients. Amsterdam *et al.* (1997) reported that decreased clinical response at higher plasma concentrations and Altamura *et al*. 1994 was find that Concentrations of fluoxetine plus norfluoxetine above 500 mg/L appear to be associated with a poorer clinical response than lower concentrations.

No significant relation has been found between fluoxetine concentration and general adverse effects in this study and this is in agreement with Beasley study in 1990 which discuses "fluoxetine: relationships among dose, response, adverse events, and plasma concentrations in the treatment of depression" and found adverse events were not related to plasma concentrations. While Altamura *et al*. reported the increase in incidence of nausea and vomiting with the increase in fluoxetine dose.

Direct relation was found between the concentration and oral adverse effects (xerostomia); the incidence of xerostomia was increased in the higher drug concentrations in our study. Also salivary flow rate showed decrement with the higher fluoxetine concentrations.

This symptom may be the result of both diminished salivary secretion and an alteration in saliva composition (Patrícia *et al*., 2012). The subjective dry mouth sensation may occur even in the presence of a normal salivary flow that is, not necessarily being associated with a diminution in the amount of saliva (Närhi, 1994).

The antidepressants inhibit the cholinergic signals in the salivary tissues and thus diminish the excretion of fluids by the glands, and interferences in central pathways (serotoninergics and dopaminergics) may also alter salivary composition (Atkinson and Baum, 2001).

It is important to emphasize that the dry mouth sensation and alteration in salivary composition may occur during periods of stress and/or acute anxiety, frequently present in depressive disorders, due to predominant stimulation of the sympathetic system, irrespective of the use of anxiolytic and/or antidepressant medication (Guggenheimer and Moore, 2003). Therefore, it may be difficult to determine whether these side effects and their intensity arise from the medical condition that led to the treatment or from the medication prescribed for it (Smith and Burtner, 1994), it probably is as a result of both (Patrícia *et al.*, 2012).

Conclusions:

SAA is a good biomarker of stress in psychological patients with a high percentage of detection comparing with the healthy levels. With the possibility of using Na^+ and K^+ level as a biomarker with SAA. The SAA level was higher in men than women.

VAS showed good correlation with the changes in the salivary flow and considered as a good subjective tool of mouth dryness. Females were more sensitive to mouth dryness than males which can be seen in the higher VAS scores reported by females. Also females were more compliant than males which can be seen from the higher percentage of male's withdrawal from the study. Higher females mean fluoxetine concentration reported in this study than males in the chronic and acute groups.

The concentration of fluoxetine in serum have no clear relations with the salivary biomarkers or with the adverse effects reported in the patient thus it cannot be used to make a decision and evaluation of the therapy and psychological situation of the patients.

Direct relation was found between the concentration and oral adverse effects (xerostomia); the incidence of xerostomia was increased in the higher drug concentrations in our study. Also salivary flow rate showed decrement with the higher fluoxetine concentrations.

Fluoxetine therapy cause **s**ignificant decline in SAA output level. And significant declinement of Na^+ level with treatment. In addition, there was a direct correlation of Na^+ with the salivary flow. Fluoxetine therapy causes significant changes in VAS score and salivary flow rate when compared with pre-therapy level.

By comparison of the concentrations of Na^+ and K^+ with the concentrations mentioned in other studies we can notice the higher concentrations of these parameters in our sample compared with others, which may be a serious or even dangerous indicator to the severe and continuous stress that our population live in. This exciting finding may give rise to many studies that should be done to investigate the effect of such acute and continuous stress on the performance and wellbeing in our population and its effect on cognitive functions and memory. Also, on the ability to make a decision of treatment. According to this point of view, that needs more studies to measure the effect of acute and chronic stress and anxiety in our population, not only to study it, but to try to find some solutions or methods to compensate or deal with it.

This is a preliminary study, limited by its sample size, but the design, findings, and inclusion of physiological measures present a contributory role in the essential line

of research. More expanded studies and researches are needed, including larger number of patients to measure the effects of continuous stress and increased levels of stress biomarker on the individuals and on cognitions and other activities in children and especially after our finding of higher levels of salivary electrolytes reported in this study. Also to measure the effects of violence, wars and post war conflicts on individuals.

Abstract

Resumen

En las últimas décadas, el estrés se ha convertido en un problema, en cierta forma inevitable, que afecta de forma negativa a la vida cotidiana. El estrés psicológico tiene un impacto negativo sobre el bienestar físico, mental y social de una persona. Se ha sugerido que puede desempeñar un papel importante en la causalidad o precipitación de multitud de problemas médicos y odontológicos que van desde graves enfermedades del corazón, cáncer, enfermedades gastrointestinales, dolores de cabeza, migrañas, ulceraciones orales recurrentes, sequedad de boca, etc.

Para entender mejor el papel del estrés en la vida de los ciudadanos y proceder a su control, es necesario disponer de métodos de medida fiables y objetivos. En la actualidad, la mayoría de los métodos de diagnóstico psicológicos utilizados consisten en cuestionarios, que por lo general recogen la percepción de los síntomas del estrés por el propio paciente. Por tanto, al tratarse de una evaluación subjetiva, proporcionan resultados muy inconsistentes, ya que los datos obtenidos dependen del estado de ánimo y la actitud de los propios pacientes. Son numerosas las personas con estrés que tienen tendencia a negar o exagerar su condición real, lo que puede conducir a una desviación en el estudio y a un error en los resultados obtenidos.

Parece clara por tanto la necesidad de encontrar indicadores biofisiológicos o biormarcadores que sean fiables, representativos y objetivos de los cambios ocurridos en el organismo como consecuencia del estado de estrés y que permitan además el seguimiento de su tratamiento.

Entre ellos, los biomarcadores salivales han sido ampliamente investigados al consistir en una medida no invasiva y sencilla, que permite una fácil recogida de muestras, si se compara con otras como la de sangre u orina. Su facilidad contribuye de manera decisiva al cumplimiento por parte de los pacientes.

En la detección del estado de estrés se han empleado como biomarcadores el cortisol, diferentes inmunoglobulinas y determinados parámetros que miden la función cardiovascular. No obstante, en diferentes estudios se ha demostrado que la medida de las concentraciones de alfa amilasa salival (SAA) muestra una elevada sensibilidad frente a los cambios en las condiciones de estrés, tanto físicos como psicológicos, por lo que podría ser considerada como un marcador prometedor en la detección y evaluación del estrés biológico y por tanto de gran valor para la investigación psicofisiológica y para la práctica clínica.

Resumen

Resulta difícil describir la situación de la población en Irak ya que no hay estadísticas precisas, especialmente en el sector de la salud, afectado dramáticamente por la guerra. El número de sujetos que necesitan en la actualidad y necesitarán en el futuro un tratamiento psicológico se ha visto incrementado debido a la tensión creciente y a la violencia resultante de los conflictos de la guerra. Por ello parece necesario encontrar un método sencillo y económico que sirva para detectar con rapidez y fiabilidad la situación de estrés de los individuos, con objeto de poder prevenir o tratar convenientemente el deterioro de su salud mental. Este es el principal objetivo de este trabajo de tesis doctoral.

Desde 2003, se ha observado un incremento significativo de la incidencia de trastornos psiquiátricos en la población iraquí, sin embargo, existe poca información fiable sobre ello. Son muy pocos los datos epidemiológicos disponibles. Por ello, otro objetivo general de esta tesis doctoral ha sido el estudio y evaluación de las condiciones psicológicas/psiquiátricas de un grupo de población representativo de la población iraquí, antes de la ocupación por parte del ISIS, pero en un momento de gran violencia y estrés.

También ha sido objetivo de esta tesis doctoral analizar y evaluar la incidencia de efectos adversos originados por los tratamientos farmacológicos, administrados a pacientes con un diagnóstico psiquiátrico preciso.

Fluoxetina fue el primer inhibidor selectivo del re captación de serotonina (SSRI) aprobado para el tratamiento de la depresión en seres humanos, siendo uno de los antidepresivos más utilizados. Se han notificado numerosos efectos adversos de tipo general y que afectan a la cavidad oral en particular. En concreto algunos de los efectos secundarios observados están relacionados con una alteración en la secreción salival y su composición.

A pesar de que fluoxetina fue aprobada por la American Food and Drug Administración (FDA) en 1987 para el tratamiento de la depresión, y luego para el tratamiento de otros trastornos psiquiátricos en adultos y niños, en Irk su introducción a la terapéutica fue más tardía, no existiendo datos fiables sobre la incidencia de efectos adversos. Probablemente esto sea debido a que la mayoría de la sociedad no reconoce estar padeciendo de trastornos psiquiátricos y depresión.

Para una gran mayoría de la población de Oriente Medio, por motivos de creencias religiosas y probablemente por miedo al rechazo social, no es aceptado el concepto de tratamiento farmacológico para un trastorno psiquiátrico, especialmente para la depresión.

Resumen

Este estudio farmacológico clínico ha sido uno de los primeros realizados en la ciudad de Mosul y probablemente en Irak sobre pacientes con alteraciones psiquiátrico/psicológicos tratados con fluoxetina, en los que se fomenta la información sobre efectos adversos intentando con ello su prevención y adecuado tratamiento.

La saliva es uno de los factores más importantes en la regulación de la salud oral. Su producción y composición se ven modificados durante el desarrollo de la enfermedad y también como consecuencia del tratamiento con fármacos SSRI. Como ya se ha comentado, en el caso de la terapéutica con fluoxetina, se han descrito diversos efectos adversos, algunos de tipo general y otros que afectan a la cavidad oral.

Entre los objetivos específicos de este estudio figura, la evaluación de la influencia de las enfermedades psiquiátricas y de su tratamiento con fluoxetina sobre el flujo salival y sobre su composición (SAA, sodio y potasio), e igualmente la evaluación de los efectos adversos (generales y u orales) del tratamiento con este fármaco y su relación con la dosis administrada y la duración del mismo.

Los objetivos específicos del estudio fueron:

- Evaluar la utilidad de la actividad alfa amilasa salival (SAA) como un marcador biológico de estrés, comprobando su eficacia como prueba diagnóstica.
- Estudiar la posibilidad de utilizar las medidas de las concentraciones de sodio y potasio salival como marcadores de estrés, estableciendo su relación con las concentraciones de SAA y volumen del flujo salival, tanto en voluntarios sanos como en pacientes psiquiátricos tratados o no con fluoxetina.
- Evaluar la incidencia de las enfermedades psiquiátricas sobre el volumen de flujo salival y composición de la saliva (SAA, Na^+, K^+).
- Estudiar la incidencia de xerostomía y su gravedad en los pacientes tratados con fluoxetina, empleando medidas objetivas y subjetivas mediante la aplicación de una escala VAS.
- Cuantificar los efectos adversos de tipo general u oral asociados al tratamiento con fluoxetina, estableciendo su relación con la dosis y duración.
- Analizar, mediante cromatografía de alta resolución (HPLC), las concentraciones plasmáticas de fluoxetina en los pacientes tratados, estableciendo su relación con las concentraciones de SAA, Na^+, K^+ y con la incidencia de efectos adversos.

Materiales y métodos:

En el estudio han participado ochenta personas divididas en dos grandes grupos:

1. Grupo de Estudio (Pacientes): consiste de (60) pacientes con edad entre los (15-56) años. Para investigar los efectos del tratamiento con fluoxetina se dividen en dos subgrupos:

1.1. Grupo de pacientes agudo: integrado por 39 pacientes (23 hombres, 16 mujeres) diagnosticados de diferentes enfermedades psicológicas y tratados con fluoxetina después de dos semanas de período de lavado. En este grupo se realizó un seguimiento durante dos meses. El promedio de edad es de (35.4±10.7) años.

1.2. Grupo de pacientes crónicos constituido por 21 pacientes (7 hombres, 14 mujeres), diagnosticados de diferentes enfermedades psicológicas y el promedio de edad es de (33.95±10.7) años. Estos pacientes fueron tratados con diferentes dosis de fluoxetina durante diferentes periodos de tiempo.

2. Grupo de Control: incluye 20 sujetos sanos, no fumadores (10 hombres, 10 mujeres) con el promedio de edad es de (35.6±13.9) años.

De cada paciente se obtuvieron muestras de saliva y sangre en cada una de las visitas realizadas: una visita en el grupo de pacientes crónicos y tres (pre-, post4- y post8-semanas de tratamiento) en el grupo de pacientes con tratamiento agudos. Además, se determinó el volumen de flujo salival. En las muestras de saliva se han analizado las concentraciones de SAA, Na^+, K^+. En las muestras de sangre se analizó cromatográficamente (HPLC) la concentración de fluoxetina.

A partir de los informes clínicos emitidos para cada uno de los pacientes, en las diferentes visitas, se recogieron datos relativos a su sintomatología y a la incidencia de efectos adversos. También en cada visita se aplicó a los pacientes la escala VAS.

Resultados y discusión:

La SAA muestra valores significativamente más altos en los pacientes con alteraciones psiquiátricas en comparación con el grupo control ($p = 0.0001$). Las diferencias encontradas entre ambos grupos permiten estimar SAA como un parámetro de alta capacidad para el diagnóstico del estrés psicológico, pues sirvió para detectar la alteración en el 76.9% de los pacientes pre-tratados y en el 85.7% de los sujetos pertenecientes al grupo pacientes crónicos. Estos porcentajes podrían incrementarse si se utilizan también las mediciones de las concentraciones de Na^+ y K^+. Los resultados indican una buena capacidad de detección y por tanto sitúan a SAA como un buen biomarcador de estrés.

Dependiendo de las respuestas fisiológicas al estrés, es posible explicar los incrementos observados en la actividad SAA. Se sabe que el estrés psicosocial provoca diferentes respuestas de los sistemas fisiológicos, en particular el aumento de las actividades en el eje hipotálamo-pituitario-adrenal (HPA) reflejada por la secreción de cortisol, así como en el sistema simpático- adrenomedular (SAM) reflejada por las concentraciones de SAA.

Se han publicado varios estudios en los que se ha investigado la actividad salival después de un estrés físico o psicológico agudo (Granger *et al.*, 2006; Nater *et al*., 2006; Malamud y Rodriguez-Chávez, 2011). Granger *et al*., (2007) indicó que "un aumento de actividad SNS conduce a un incremento en la producción de SAA y por ello dicho aumento puede evaluarse examinando muestras de saliva". Varios estudios han analizado la relación entre la norepinefrina y SAA ya que ambos están relacionados con la activación del SNS. Sin embargo, los resultados han sido inconsistentes (Chatterton *et al*., 1996; Nater *et al*., 2006).

Allwood *et al*., (2011) encontraron que la SAA es más reactiva a estresores experimentales que el cortisol.

Este es el primer estudio en el que se evalúa la actividad SAA en pacientes psiquiátricos en fase aguda y crónica, y sometidos a diferentes situaciones psicológicas, estableciendo su relación con las concentraciones de electrolitos salivales y con los efectos adversos identificados en los pacientes. El tamaño de muestra es pequeño para que pueda representar a la población en general, pero puede dar una idea acerca de los cambios que ocurren en la saliva como consecuencia del estrés y otras condiciones psicológicas. Por ello consideramos que sería necesario realizar nuevas investigaciones, con un mayor número de pacientes, para confirmar los resultados obtenidos.

Los cambios observados en este estudio se detallan a continuación.

En el grupo pre-tratado (enfermos) se ha percibido un aumento significativo de SAA y de la concentración de Na^+, y una reducción en las concentraciones de K^+, en comparación con el grupo control de voluntarios sanos ($p<0.05$). También se ha observado una reducción significativa del volumen de flujo salival (FR) y un incremento en los valores de VAS en comparación con el grupo control ($p<0.05$). En el grupo de pacientes pre-tratados se detectó un elevado nivel de nerviosismo, dolor de cabeza, insomnio, modificación en el apetito, xerostomía y dysgeusia. Las diferencias en la incidencia de efectos adversos respecto al grupo de voluntarios sanos fueron estadísticamente significativas ($p < 0.05$).

Como se ha comentado anteriormente, la liberación de SAA está regulada por el sistema nervioso autónomo (Bagan-Sabastain, 2004) por lo que su incremento puede relacionarse con un incremento en la actividad simpática (Granger *et al*., 2006; 2007). La actividad SAA aumenta, como parte de la respuesta normal del organismo al estrés, mediada por el sistema simpático adrenomedular. Por ello algunos autores consideran SAA como un buen biomarcador para evaluar dicha actividad simpática (Friedlander y Mahler, 2001).

Por otra parte, en los enfermos psiquiátricos, se ha percibido un incremento significativo ($p < 0.05$) en las concentraciones salivales de Na^+ y una reducción también significativa de las concentraciones de K^+, en comparación con el grupo control. Esto podría ser debido a que la mayoría de los pacientes pre-tratados ya sufren de depresión y probablemente de otros trastornos psiquiátricos relacionados. Es conocido que la depresión puede asociarse a un incremento en la producción de aldosterona lo que incrementa la reabsorción de Na^+ y el intercambio de K^+. Esto podría explicar el mayor aumento en las concentraciones salivales de Na^+ y en la disminución en las concentraciones de K^+ en los enfermos psiquiátricos cuando se compara con individuos sanos (Zanatta *et al.*, 2001).

En el grupo de pacientes crónicos, el tratamiento con fluoxetina indujo una disminución significativa ($p < 0.05$) en los valores de SAA, respecto al grupo de pacientes pre-tratados. Por el contrario, las concentraciones de Na^+ fueron significativamente menores y las de K^+ mayores, respecto al mismo grupo de pacientes (pre-tratados). También se observaron modificaciones significativas en el volumen de flujo salival y en los valores de VAS respecto al grupo correspondiente a los enfermos antes del tratamiento.

En el grupo de los pacientes agudos o de pacientes con seguimiento en el tratamiento con fluoxetina durante dos meses, se han observado diferencias significativas en los valores de SAA entre las distintas lecturas (pre, post1, post2). Aunque SAA disminuye con el tratamiento, se mantiene en valores más elevados que en el grupo control de voluntarios sanos.

Por tanto, se observa que la concentración de SAA es significativamente mayor en enfermos (representados por los grupos de agudos y crónicos) que en los individuos sanos integrados en el grupo control. La medida de la actividad SAA fue significativamente mayor en el grupo pre-tratado (19.87 u/min) que en el post1 (12.86 u/min) y post2 (11,43

u/min), lo que puede indicar la eficacia del tratamiento con fluoxetina para reducir el estrés psicológico.

Estos resultados están en consonancia con lo publicado por Noto *et al.*, (2005) que propone como indicador útil para detectar estrés psicológico, la medida de SAA.

Las modificaciones en los niveles de SAA observados podrían estar relacionados con la activación del sistema beta-adrenérgico y en base a ello con el estrés psicológico y otros desórdenes psiquiátricos. Apoyando esta hipótesis, Kivlighan y Granger, (2006) e Inagaki *et al*., (2010) indican el papel predominante del sistema nervioso simpático en la secreción de SAA bajo condiciones de estrés psicológico y la no influencia del sistema parasimpático.

La respuesta de los pacientes al tratamiento con fluoxetina, debida al incremento de las concentraciones de 5-HT, induce una mejoría en la enfermedad depresiva y otras alteraciones psicológicas, reduce el estrés psicológico y mejora en general, el estado de ánimo de los pacientes. Por tanto, la disminución en la secreción de SAA observada en los pacientes podría reflejar una disminución en la actividad simpática.

En cuanto a las concentraciones de Na^+, también se observaron cambios significativos ($p < 0.05$) entre los pacientes pre- y post-tratados con fluoxetina. Por el contrario, no se observaron cambios estadísticamente significativos en las concentraciones de K^+. Los cambios observados fueron una disminución en las concentraciones de Na^+ y un incremento en las de K^+ en los pacientes tratados con fluoxetina.

Los estudios publicados no ofrecen una explicación científica para las modificaciones observadas en las concentraciones de estos iones en la saliva de los pacientes psiquiátricos tratados con fluoxetina. Investigaciones realizadas en enfermos no psiquiátricos sí podrían justificar en parte nuestros resultados. Se ha observado que un incremento del volumen de flujo salival está relacionado con incrementos en las concentraciones de proteína total, Na^+, calcio, cloruros y bicarbonato, así como en el pH (Edgar, 1992; Tenovuo *et al*., 1994). La disminución en el volumen del flujo salival ocasionado por el tratamiento con fluoxetina, ampliamente documentado, podría explicar la disminución de las concentraciones de Na^+ observadas en los pacientes tratados, alcanzando en el grupo post2 de tratamiento niveles similares a los encontrados en la saliva de los voluntarios sanos. Estos resultados están de acuerdo con los encontrados por Höld *et al*., (1999) al proponer una relación directa entre la secreción de Na^+ y el incremento del flujo salival e indirecta respecto a las concentraciones de K^+. También pudiera deberse a una modulación de tipo hormonal que incidiría de manera directa sobre la reabsorción

de Na^+ y la secreción de K^+ (Wotman *et al.,* 1973; Kakmoto *et al.,* 1988); o bien al efecto de la fluoxetine como inductor de la actividad ATPase Na-K (27% respecto al control) lo que provocaría igualmente un incremento en la reabsorción de Na^+ y en la secreción de K^+ (Zanatta *et al*., 2001).

Conforme a lo anterior, las importantes diferencias encontradas en este trabajo en las concentraciones de Na^+ y K^+ respecto a las mencionadas en otras investigaciones, podrían ser indicadoras del estrés grave y continuado al que está sometido la población iraquí. Este interesante hallazgo podría dar lugar al inicio de nuevos estudios dirigidos a investigar el efecto del estrés agudo y continuo en el rendimiento y bienestar de la población, y su efecto sobre las funciones cognitivas y la memoria. También podrían ser el punto de partida para avanzar en el abordaje de su control y tratamiento.

En cuanto a la evaluación de la seguridad y tolerabilidad de los tratamientos, 16 pacientes (41.02%) del grupo de tratamiento agudo, tuvieron que abandonar el estudio. 8 pacientes (20.51%) debido a efectos adversos como trastornos gastrointestinales (GIT), insomnio, disfunción sexual y problemas de la articulación temporomandibular (TMJ); y 8 pacientes (20.51%) debido a la falta de eficacia y no cumplimiento terapéutico. Los principales efectos adversos causados por la terapia con fluoxetina fueron dificultades respiratorias, sueños anormales, somnolencia, insomnio, dolor de cabeza, pérdida de peso, cambios en el apetito, temblor, mialgias, anorexia, náuseas, diarrea, ansiedad, cambio en el sentido del gusto (disgeusia), xerostomía y problemas de la TMJ.

La influencia del género sobre los parámetros estudiados solo se evidenció en cuanto a los valores de concentración de Na^+, significativamente más elevados en mujeres que en hombres en el grupo de enfermos crónicos. Mientras que los efectos adversos de tipo general como ansiedad, dolor de cabeza y nerviosismo fueron significativamente mayores en hombres que en mujeres en el mismo grupo de pacientes. En el grupo de pacientes con tratamiento agudo la incidencia de ansiedad, estreñimiento y palpitaciones fue significativamente mayor en varones que en hembras.

En cuanto a las manifestaciones que afectan a la cavidad oral, solo en el caso de la disgeusia, la incidencia ha sido significativamente mayor en hombres que en mujeres en el grupo crónico, mientras que en el grupo de pacientes con tratamiento agudo han sido los trastornos de la articulación temporomandibular los que se han manifestado en un mayor número de casos también en hombres que en mujeres.

En relación a la influencia de la dosis administrada de fluoxetina, se ha observado que las concentraciones de Na^+ en saliva y la incidencia de nerviosismo han mostrado un

aumento significativo con la dosis de 40 mg/d en el grupo de pacientes crónicos ($p = 0,04$ y $p = 0.034$ respectivamente). En el grupo de pacientes agudos, sólo se observó un incremento significativo en la incidencia de ansiedad cuando la dosis administrada fue de 60 mg/d ($p = 0,035$).

La duración del tratamiento también influyó en los parámetros analizados en este trabajo de investigación. El flujo salival (FR) disminuyó con la duración del tratamiento mientras que los valores subjetivos obtenidos mediante la escala VAS fueron mayores en caso de tratamientos prolongados. En cuanto a la aparición de efectos adversos, los tratamientos de mayor duración se asociaron a una significativa mayor incidencia de pérdida de peso, mialgia, estreñimiento, palpitaciones, dificultad respiratoria, nerviosismo, ansiedad, insomnio, nausea, disgeusia, xerostomía, trastornos de la articulación temporomandibular y lengua negra vellosa ($p < 0.05$).

En los pacientes tratados con antidepresivos siempre se debería realizar un seguimiento médico con objeto de poder detectar un posible empeoramiento clínico, tendencias suicidas o cambios inusuales en el comportamiento, especialmente durante los primeros meses de tratamiento, o cuando se procede a modificar el mismo, aumentando o disminuyendo la dosis. Por ello, en este trabajo se procedió a cuantificar las concentraciones plasmáticas del fármaco (fluoxetina) durante las diferentes visitas de los pacientes, empleando métodos cromatográficos (HPLC).

Son varios los factores que pueden influir en las concentraciones plasmáticas de los fármacos, entre ellos modificaciones en la absorción, distribución y aclaramiento de los mismos, que pueden verse afectados por aspectos genéticos y por diferencias interindividuales. Los resultados obtenidos, aunque variables, se encuentran dentro de los márgenes terapéuticos estimados para fluoxetina.

En general, las concentraciones medias fueron más elevadas en mujeres que en hombres en los dos grupos de tratamiento (agudo y crónico). Estos resultados están en consonancia con los obtenidos en trabajos previos, en los cuales se demostró que, a igual dosis, las concentraciones plasmáticas de fluoxetina y norfluoxetina son más bajas en hombres (Amsterdam *et al.,* 1997; Ferguson, 2006; Blazquez *et al*., 2014). Por el contrario, Pato *et al.,* (1991) indica que el sexo y la edad de los pacientes no influyen en la concentración plasmática ni en el metabolismo de la fluoxetina. En este estudio, no se observó una relación clara entre la concentración plasmática y el abandono del estudio por algunos pacientes, sin embargo, en éstos se encontraron concentraciones plasmáticas del fármaco más elevadas en comparación con otros pacientes. Por otra parte, Amsterdam

et al., (1997) indicaron que la respuesta clínica disminuía cuando las concentraciones plasmáticas eran más elevadas y Altamura *et al*., (1994) que cuando estas concentraciones plasmáticas de fluoxetina y norfluoxetine eran superiores a 500 mg/L se obtenía peor respuesta clínica que a concentraciones más bajas.

En este estudio, no se ha encontrado ninguna relación significativa entre la concentración plasmática de fluoxetina y la aparición de efectos adversos de tipo general. Estos resultados están de acuerdo con los obtenidos por Beasley en el año 1990 en su estudio titulado "Fluoxetina: relaciones entre dosis, respuesta, efectos adversos y concentraciones plasmáticas, en el tratamiento de la depresión" en el cual tampoco se pudo encontrar una relación entre las concentraciones plasmáticas del fármaco y la incidencia de efectos adversos. Por el contrario, Altamura *et al.*, (1994) observaron una mayor incidencia de náuseas y vómitos al incrementarse las concentraciones plasmáticas de fluoxetina.

En cuanto a los efectos adversos relacionados con la cavidad oral, se ha encontrado una relación directa entre la concentración plasmática y la incidencia de los mismos. Se ha observado un mayor número de casos de xerostomía y disminución del flujo salival cuando las concentraciones plasmáticas son más elevadas. Este síntoma puede ser el resultado de una disminución en la secreción de la saliva y de una alteración en su composición (Patricia *et al*., 2012). No obstante, la sensación subjetiva de sequedad de boca puede ocurrir incluso cuando el flujo de saliva es normal, por tanto, no está necesariamente asociada a una disminución de su cantidad (Närhi, 1994).

Los antidepresivos inhiben las señales colinérgicas en los tejidos salivales induciendo una disminución en la secreción de fluidos por las glándulas. Además, al incidir sobre las vías de señalización serotoninérgicas y dopaminérgicas, pueden modificar su composición (Atkinson y Baum, 2001).

Es importante destacar que la sensación de sequedad de boca y la modificación de la composición de la saliva puede ocurrir también durante períodos de estrés y/o ansiedad aguda, frecuentes en estados depresivos, como consecuencia de la estimulación predominante del sistema simpático, e independientemente del uso de medicamentos ansiolíticos o antidepresivos (Guggenheimer y Moore, 2003). Por lo tanto, resulta difícil determinar si estos efectos secundarios y su intensidad son debidos a la situación de estrés psicológico o enfermedad que motivó el tratamiento; son debidos a la medicación prescrita

para resolverlos (Smith y Burtner, 1994), o lo que es más problable, sean el resultado de ambos (Patrícia *et al.*, 2012).

Teniendo en cuenta los resultados de este estudio y la discusión de los mismos, se pueden obtener las siguientes **conclusiones**:

1. La evaluación de la cantidad y el análisis de la composición de la saliva podrían ser considerados como un método analítico, no invasivo y eficaz, para el estudio de los cambios originados en el estado de los pacientes psiquiátricos, así como en la evolución de su tratamiento.
2. Se han encontrado diferencias significativas en la concentración salival de SAA, Na^+ y K^+ entre los voluntarios sanos, los pacientes con alteraciones psicológicas, y los pacientes tratados farmacológicamente.
3. La medida de la concentración de alfa amilasa salival (SAA) puede considerarse un adecuado biomarcador para evaluar el estrés en pacientes con alteraciones psicológicas. También puede ser empleado para evaluar la eficacia de los tratamientos farmacológicos sobre el grado de estrés de los pacientes.
4. A pesar de que el tratamiento con fluoxetina origina una disminución significativa en la liberación de SAA y en la concentración de Na^+, no es posible establecer una clara correlación entre su concentración plasmática, determinada en los pacientes tratados, con los biomarcadores de estrés y/o con la incidencia de efectos adversos. Por ello, no es posible considerar la medida de la concentración plasmática de fluoxetina, como estimador para el seguimiento de la evolución del estado psicológico de los pacientes, ni para la toma de decisión en el tratamiento.
5. Se ha observado una correlación directa entre la concentración de Na^+ y la medida del flujo salival.
6. Los resultados obtenidos, tras la aplicación a los pacientes de la escala analógica visual (VAS), ha mostrado una buena correlación con los cambios observados en el flujo salival. En pacientes enfermos, valores elevados en VAS están relacionados con una disminución en el flujo de saliva. Tras el tratamiento con fluoxetina se observan cambios significativos en los valores de VAS y en el flujo salival. Por ello, esta escala puede ser considerada una buena herramienta para la estimación subjetiva de la percepción de sequedad de boca.

7. En este estudio el porcentaje de abandono del tratamiento farmacológico se ha estimado en un 41.02%, de los cuales un 20.5% ha sido consecuencia de los efectos adversos provocados por el tratamiento, especialmente trastornos gastrointestinales, insomnio y alteraciones en la función sexual. El abandono del estudio como consecuencia de alteraciones en la articulación temporo-mandibular (TMJ) ha sido del 2.56%. Xerostomia y disgeusia son efectos adversos asociados al tratamiento con fluoxetina.
8. El género influye significativamente tanto en el abandono del tratamiento como en la incidencia o percepción subjetiva de efectos adversos. Se observó un mayor número de abandonos en los varones. Sin embargo, en el grupo de mujeres, los valores de VAS fueron más elevados, por lo que parecen ser más sensibles a la percepción de sequedad de boca.
9. Se constata la escasa información científica publicada acerca de los efectos adversos originados en la cavidad oral por los tratamientos con antidepresivos y en especial por inhibidores en el re captación de serotonina (SSRIs), así como sobre su posible prevención y tratamiento. Para mejorar ese conocimiento se proponen las siguientes recomendaciones:
    - En los registros del seguimiento diario de los pacientes con alteraciones psiquiátricas debería incluirse información sobre la higiene de la cavidad oral y los efectos de la medicación sobre la misma.
    - Sería conveniente alentar a los pacientes para que se preocupen por la higiene y salud de su cavidad bucal como parte importante de su estado saludable o como manifestación de algún problema psiquiátrico. También fomentar en ellos la comunicación de cualquier incidencia al médico.
    - Crear programas educativos dirigidos a los ciudadanos para controlar el estrés y para manejar convenientemente las consecuencias psíquicas y físicas del mismo.
    - Implementar programas de formación continua para profesionales sanitarios que les permita la actualización de conocimientos sobre los síntomas orales, tanto de los trastornos psiquiátricos como de los efectos adversos inducidos por los tratamientos.

1. INTRODUCTION

1. INTRODUCTION

1.1 Stress and their developed biological markers

In the last recent decades, stress has become an inevitable part plaguing the daily lives. Psychological stress has a negative impact over physical, mental and social well-being of a person. It has been suggested to play an important role in causation or precipitation of multitude of medical and dental problems ranging from serious heart diseases, cancers, gastrointestinal diseases, to common headaches, migraine, recurrent oral ulcerations, burning and dry mouth.

In order to better understand the role of stress, valid and reliable measurement of stress is of utmost importance. Since, most of methods used for the diagnosis of stress are questionnaires form (subjective methods) which depends on person and there is many inter individual variation between subjects, thus there is increased need of biological stress markers to provide an objective, reliable and authentic evidence of stress. Many biomarkers have been used in determination of stress such as cortisol levels, immunoglobulins, chromogranin-A, cardiovascular parameters. Studies showed there is an evidence of sensitivity of SAA (salivary alpha amylase) levels in response to different stressful conditions, physically and psychologically.

Salivary biomarkers are important field of research because of easy, non-invasive and rapid collection of samples compared to the blood and urine samples thereby increasing the patient compliance. The components of saliva act as a “mirror of the body 's health”, and the widespread use and growing acceptability of saliva as a diagnostic tool is helping individuals, researchers, health care professionals and community health programs to better detect and to monitor diseases and to improve the general health of the public (Dodds and Johnson, 2005). Many studies showed that analysis of saliva sample is a convenient means for assessment of physiological conditions, evaluation the serum concentration of medicine and assessment of the severity of an illness (Loewit *et al.*, 1996; Tadasi, 2002).

Psychosocial stress is widely known to induce various adaptational responses of physiologic systems with particular increasing activities in the hypothalamus-pituitary-adrenal axis (HPA) as well as in the sympathetic-adrenal-medullary (SAM) system. Cortisol levels reflect the HPA activity whereas SAA is said to reflect the SAM activity. Many studies comparing SAA activity with stress and/or adrenergic activity had shown that SAA reflected the adrenergic activity and thus might be used as a reliable index of the SAM activity during stress. Chatterton and colleagues (Chatterton *et al.*, 1996; 1997)

linked levels of SAA to sympathetic activation during physically and psychologically stressful conditions which found to be closely associated with changes in norepinephrine concentrations. Psychological stress activates both the HPA axis, as well as the SAM axis, which manifests as changes in cortisol and SAA output. For example, SAA has been found to respond to psychological stress (Bosch *et al*., 1996; Skosnik *et al*., 2000; Nater *et al*., 2005, 2006; Rohleder *et al*., 2006). Sympathetic stimulation causes high SAA release from the parotid and submandibular acinar cells, whereas parasympathetic stimulation causes low SAA release from the sublingual acinar cells (Kelly *et al.*, 2010).

Bosch, in his study in 1996, measured the salivary proteins associated with stress and found significant stress-mediated increase of salivary total protein concentration, alpha-amylase activity, amylase/protein ratio, alpha-amylase output, s-IgA concentration, and s-IgA output. While Chatterton *et al.* (1997) identified that salivary α-amylase levels increased in response to exercise. These authors compared levels of salivary α-amylase in males prior to and following exercise, a written examination or rest, and identified that aerobic exercise induced a three-fold mean increase in α-amylase levels.

Nater and colleagues had done many studies about SAA. In 2005 they set out to investigate human salivary alpha-amylase changes employing a reliable laboratory stress protocol to investigate the reactivity of salivary alpha-amylase to a brief period of psychosocial stress. And they report significant differences between psychosocial stress and the rest condition in alpha-amylase activity, cortisol levels and heart rate, with marked increases before and after stress. While the author said in another study published in 2006 that salivary alpha-amylase is sensitive to psychosocial stress. Since it does not seem to be closely related to other biological stress markers such as catechol amines and cortisol, salivary alpha-amylase may be a useful additional parameter for the measurement of stress.

These studies and others showed there is an evidence of sensitivity of SAA levels in response to different stressful conditions, physically and psychologically. Also some studies provide evidences about the relation of salivary sodium and potassium and the physical and psychological stressor.

Changes in SAA are thought to have implications for health. And consider a promising role of salivary alpha amylase as a possible biological stress marker.

1.2 Mental health problems

Mental health problems are a growing public health concern. A recent index of 301 diseases found mental health problems to be one of the main causes and a major contributor of the overall disease burden worldwide. (Prince *et al.*, 2007; Mark *et al.*, 2009; Vos *et al*., 2013). Costs of mental health services are high and yet there is evidence that the coverage of mental health care is insufficient to address current need (Rice and Miller, 1995; Ferrari *et al*., 2013). Mental health problems constitute the largest single source of world economic burden, with an estimated global cost of £1.6 trillion (or US$2.5 trillion) – greater than cardiovascular disease, chronic respiratory disease, cancer, and diabetes on their own (Insel, 2011).

According to the World Health Organization (WHO, 2008), major depression is forecast to be the condition causing the second highest loss in disability adjusted life years by the year 2020. Depression has the third highest burden of all diseases global. WHO estimates that depression will be the number one health concern in both the developed and developing nations by 2030 (WHO, 2008).

According to the 2010 Global Burden of Disease Study, mental health and behavioral problems (e.g. depression, anxiety and drug use) were reported to be the primary drivers of disability worldwide, causing over 40 million years of disability in 20 to 29-year-olds (Lozano *et al*., 2012). The most predominant mental health problems worldwide are depression and anxiety (White ford *et al*., 2013). Also they found major depression to be the second leading cause of disability worldwide and a major contributor to the burden of suicide and ischemic heart disease (Ferrari *et al*., 2013). Globally, up to 90% of people diagnosed with anxiety and depression are treated in primary care. However, there are many individuals who are undiagnosed and therefore do not seek treatment (NICE, 2011).

1.2.1 Depression, prevalence, historical reviews

Depression is a broad and heterogeneous diagnosis, characterized by depressed mood and/or loss of pleasure in most activities. Severity of the disorder is determined by both the number and severity of symptoms and the degree of functional impairment.

Because depression displays high rates of lifetime prevalence, early age of onset, high chronicity, and role impairment, the WHO has ranked depression as the single most burdensome disease in the world in terms of years lived with disability (Murray and

Lopez, 1996; Richards, 2011; WHO, 2012; Ferrari *et al*., 2013). Depression is one of the leading causes of disease worldwide. Historically conceived as either a disease of the mind or of the brain, treatment options followed this etiology. Current diagnostic assessment of depression is based on descriptions of symptoms, their presence and magnitude over time. Epidemiological studies demonstrate that depressive disorders are highly prevalent: displaying high rates of lifetime incidence, early age onset, high chronicity, and role impairment.

Depression is one of the most commonly diagnosed mental disorders among adults. Our understanding of the course and nature of depression has changed significantly in the last twenty years. From being seen as an acute and self-limiting illness, to a growing clarity that for many depressions is now considered a chronic, lifelong illness (WHO, 2012).

Prevalence of depression is of concern, as the cost that depression exacts is considerable. It is not only economically detrimental, but also engenders significant personal and interpersonal suffering alongside its societal impact (Johnson *et al*., 1992). In the U.S. for the year 2000 the economic burden of depression was estimated at $83.1 billion. Thirty-one percent (31%) related to direct medical costs, 7% related to mortality costs, and 62% to workplace costs (Greenberg *et al*., 2003). In Europe for the year 2004 the annual cost of depression was estimated at €118 billion. Direct healthcare costs amounted to 36% and indirect costs due to morbidity and mortality amounted to 64% (Sobocki *et al*., 2006). In 2012, depression is estimated to affect 350 million people. The World Mental Health Survey conducted in 17 countries found that on average about one in 20 people reported having an episode of depression in the previous year (WHO, 2012).

Historically, mood disorders usually diagnosed depending on the Diagnostic and Statistical Manual of Mental Disorders which put definitions and criteria for diagnosis of psychiatric disorders in different editions. In the Diagnostic and Statistical Manual of Mental Disorders-First Edition (DSM-I) mood disorders have been conceived as either "organic" or "reactive," (DSM-I) (American Psychiatric Association [APA], 1952). The second edition of the manual (DSM-II) continues this basic distinction using the terms "psychotic" and "neurotic." Mood disorders were understood as either a disease of the brain and organic, or neurotic and therefore a disease of the mind ((DSM-II) (American Psychiatric Association, 1968; Boland and Keller, 2002). Disorders of a neurotic or reactive variety could be cured once the cause was removed. Those of a psychotic or organic nature were viewed as having a less favorable outcome. Considered chronic, their

fate was institutionalization combined with somatic treatment. The third Edition (DSM-III) (American Psychiatric Association, 1980) favored a descriptive approach, whereby individuals were diagnosed with a mood disorder based on whether or not they met clear diagnostic criteria, which was based on a constellation of symptoms and specific duration. The Fourth Edition (DSM-IV-TR) describes a Major Depressive Disorder (MDD) diagnosis based on the presence of a specified number of symptoms with a precise duration. Primarily symptoms of either depressed mood or loss of interest or pleasure are present. Additionally, the criteria of at least five items from the DSM-IV-TR (APA, 2000) list need to be present for a duration of two weeks and as such, represents a change from previous functioning. It includes depressive mood and loss of interest in most activities, appetite and sleep disturbance, feelings of worthlessness and guilt, suicidal thoughts and ideation (American Psychiatric Association, 2000). For DSM-V the criteria for diagnosis of these disorders remains the same as DSM-IV-TR (American Psychiatric Association, 2000). The DSM-V proposes additional diagnostic categories such as mixed Anxiety/Depression and also integrates childhood and adolescent psychiatric disorders into relevant chapters (American Psychiatric Association, 2013).

The World Health Organizations (WHO) International Classification for Diseases and Related Disorders (ICD-10) describes the criteria for a depressive episode, where at least four items, such as loss of interest in activities, lack of emotional reactions, sleep disturbance, loss of appetite, motor retardation, weight loss, loss of libido, and decreased energy are present for a duration of two weeks (World Health Organization WHO, 1993).

The goal of treatment was symptom reduction or extinction if possible. However, treatment outcomes including continued relapse and recurrence posed challenges to developing adequate treatments. (Angst, 1986; Andrews *et al.*, 2011).

1.2.2 Managements

Depression may disrupt work, family, and personal life. Many of these consequences, however, are avoidable. Depression is a treatable disease, yet many people who are depressed do not seek treatment. Depression is a disorder that can be reliably diagnosed and treated in primary care by general practitioners (The World Health Report, 2001). Despite it being a common and debilitating mental disorder, depression is clinically under-recognized and undertreated. 30-50% of cases of depression are not detected in medical settings (Anderson *et al.*, 2008).

Treatment often involves a combination of different therapies such as medication, psychological therapies, social support, and self-help techniques. An individual's treatment will depend on the severity of their symptoms. And according to WHO Guide, preferable treatment options consist of basic psychosocial support combined with antidepressant medication or psychotherapy, such as cognitive behavior therapy, interpersonal psychotherapy or problem-solving treatment. Antidepressant medications and brief, structured forms of psychotherapy are effective. Antidepressants can be a very effective form of treatment for moderate-severe depression but are not the first line of treatment for cases of mild or sub-threshold depression (Andrews *et al.*, 2011).

1.3 Antidepressants

Antidepressants are recommended by the National Institute for Health and Care Excellence (NICE) as a first-line treatment of severe depression and for the treatment of mild-to-moderate depression that persists after conservative measures such as cognitive therapy (NICE, 2009).

The different types of antidepressants are generally categorized by which natural chemicals they affect in your brain to help change your mood. They can also be broken down into categories of older and newer drugs (Bartha, 1999; Linardatos *et al*., 2008; Canadian Mental Health Association, 2010)

- Older
  Monoamine oxidase inhibitor (MAOIs) (the first antidepressants)
  Cyclics (or tricyclic antidepressants: TCAs)

- Newer
  Selective Serotonin Reuptake Inhibitors (SSRIs)
  Serotonin and Norepinephrine Reuptake Inhibitors (SNRIs)
  Norepinephrine and Dopamine Reuptake Inhibitors (NDRIs)

Medications are often increased gradually to optimize their effects. The first weeks may be more about managing any side effects rather than real symptom relief, which can take a while to begin for some. (Bartha, 1999).

1.3.1 Monoamine oxidase inhibitors (MAOIs): MAOIs work through a chemical reaction with monoamine oxidase A and B, enzymes which oxidize specific monoamines. When an MAOI reacts with monoamine oxidase, the enzyme is permanently deactivated and cannot function until the protein is replaced by the body. MAOIs are no longer used as front-line treatment for depression (the most commonly still in use today are Isocarboxazid and Phenelzine) is due to the fact that MAO is responsible for breaking down other trace dietary amines, such as tyramine and tryptophan causing alarming issues such a major hypertensive crisis or serotonin syndrome which can be fatal leading to death attributed to dietary amine consumption. As a result of these dangers and the requirement of a specific, restrictive diet, MAOIs are currently only used for patients who do not respond to other types of antidepressants (Rang, 2003; Linardatos *et al*., 2008).

1.3.2 Tricyclic antidepressants (TCA): TCAs are the oldest class of antidepressants and were used as first-line treatment for depression since the 1950's. They are characterized by three carbon rings in their molecular structure. Amitriptyline is the most commonly prescribed TCA and is the most effective for treating depression. TCAs act as ligands for the transporters and prevent them from bonding to anything else. They have a very high affinity for serotonin and norepinephrine but not dopamine. They also have a high affinity for H1 and H2 histamine receptors, and for the muscarinic acetylcholine receptor which results in TCAs also acting as effective antihistamines and anticholinergics. These affinities create undesirable side effects such as lethargy, ataxia, dry mouth, increased body temperature, tachycardia, and in severe cases delirium. These unpleasant and in some cases severe side effects are a reason TCAs have been supplanted by newer drugs for the treatment of depression. The primary reason TCAs are no longer used as first-line treatment is due to their high potential toxicity and consequently their potential to cause a fatal overdose. They are rapidly absorbed into the bloodstream as onset of overdose symptoms occurs quickly. Death is most commonly caused by the cardiac effects of acetylcholine antagonism. TCAs have been attributed to as many as 33% of all fatal poisonings, and 95% of deaths related to antidepressants alone. These dangers are significant and thus TCAs are only administered to patients who do not respond to other antidepressant medications. TCAs have been largely supplanted by SSRIs for first line treatment (Rang, 2003).

1.3.3 Selective serotonin reuptake inhibitors (SSRIs): SSRIs (fluoxetine, fluvoxamine, sertraline, paroxetine, citalopram, escitalopram) are a newer class of antidepressants which are the most widely prescribed antidepressant. They are almost exclusively used as first-line treatment for major depressive disorder. SSRIs' mode of action is to inhibit the re-uptake of serotonin into the presynaptic cell by bonding to serotonin transporter. This is different from the TCA drugs because SSRIs have high bonding affinity only for serotonin transporter and very minimal affinity to other monoamine transporters. This means that SSRIs have much more limited side effects and do not have any of the anticholinergic or antihistamine effects that TCAs do (Anderson, 2000)

SSRIs are therefore much less toxic than TCAs and are very difficult to achieve a dangerous reaction to an overdose. The most severe complication arising from overdose is serotonin syndrome, an excess of serotonin in the brain which can, in very severe cases, lead to seizures, coma, and death. SSRIs do have some minor side effects mostly related to depression itself. The most common reason for discontinuation due to side effects is sexual dysfunction. This is thought to be caused by SSRIs creating chemical imbalances in the brain in the dopamine pathways which directly influence sexual arousal and function.

SSRIs were the first type of psychoactive drug to be rationally designed; as opposed to the trial-and-error used up to that point. This involves creating a molecule that has specific binding properties based on hypothetic benefits. In the case of SSRIs, the goal was to create a molecule that bonded to serotonin transporter. This is most commonly done using computer assisted tools and analysis (Rang, 2003).

1.3.4 Serotonin and norepinephrine reuptake inhibitors (SNRIs): SNRIs (venlafaxine, milnacipram, duxoletine) are a more recent development than SSRIs, and are also very widely used. The most commonly prescribed is Venlafaxine and their metabolites. They have been shown to have slightly higher efficacy than SSRIs due to their dual mode of action, blocking both serotonin and norepinephrine transporter. SNRIs have the same sexual side effects that SSRIs do, although with lower occurrence (Kajdasz *et al.,* 2008).

1.3.5 Norepinephrine and dopamine reuptake inhibitors (NDRIs): NDRIs act on norepinephrine and dopamine transporter and thus increases the amount of both neurotransmitters in the postsynaptic cell. This is the only class of non-serotonergic drug

commonly prescribed for depression. Bupropion, has been shown to be more effective than SSRIs on its own and is also used to augment the performance of an SSRI. It does not cause sexual dysfunction or weight gain and has only isolated side effects. This indicates that sexual dysfunction is a function exclusively of serotonin reuptake inhibition, as drugs which do not effect it does not have sexual side effects (Costa *et al.*, 2002).

1.3.6 Noradrenergic and specific serotonergic antidepressants (NaSSA): This class of atypical antidepressants is solely composed of Mirtazapine. Its mode of action is completely different from the reuptake inhibitors and instead is a highly potent antagonist of H1, a histamine receptor, the serotonin receptors 5-HT2A, 5-HT2C, 5-HT3, and the alpha2-adrenergic receptor. It has no affinity for any neurotransmitter transporter. The antidepressant effect comes primarily from the antagonism of 5-HT2C which normally prevents dopamine from being released in the pleasure centers of the brain.

Mirtazapine therefore has none of the negative side effects related to increased amounts of serotonin, dopamine, or norepinephrine, instead making the available amount more effective.

It is sometimes used in addition to another antidepressant in order to alleviate their side effects and improve their antidepressant effect (Timmerman *et al*., 1998; Linardatos *et al.*, 2008).

1.4 Selective serotonin reuptake inhibitors (SSRIs)

Dysfunction of serotonergic neurotransmission is considered as one of the major underlying cause of mood and other neuropsychiatric disorders (Souza *et al.*, 2004).

The antidepressants known as selective serotonin reuptake inhibitors (SSRIs) have become widely used to treat major depression and many other psychiatric disorders (Brunton and Parker, 2008). However, the efficacy of SSRIs in depression is no greater and their onset of action is no more rapid than that of the MAOIs or TCAs. In addition, they are not completely devoid of side effects but, SSRIs are used as a first-line treatment for depression, in part because of their relatively benign adverse effect profile and safety in overdose, especially compared with the older TCAs and MAOIs (Mourilhe and Stokes, 1998; Chantal and Mike, 2007).

SSRIs are believed to increase the extracellular level of the neurotransmitter serotonin by inhibiting its reuptake into the presynaptic cell, increasing the level of serotonin in the synaptic cleft available to bind to the postsynaptic receptor.

In the brain, messages are passed from a nerve cell to another via a chemical synapse, a small gap between the cells. The presynaptic cell that sends the information releases neurotransmitters including serotonin into that gap. The neurotransmitters are then recognized by receptors on the surface of the recipient postsynaptic cell, which upon this stimulation, in turn, relays the signal. About 10% of the neurotransmitters are lost in this process; the other 90% are released from the receptors and taken up again by monoamine transporters into the sending presynaptic cell, a process called *reuptake*. SSRIs inhibit the reuptake of serotonin. As a result, the serotonin stays in the synaptic gap longer than it normally would, and may repeatedly stimulate the receptors of the recipient cell as shown in figure (1.1).

In the short run, this leads to an increase in signaling across synapses in which serotonin serves as the primary neurotransmitter. On chronic dosing, the increased occupancy of pre-synaptic serotonin receptors signals the pre-synaptic neuron to synthesize and release less serotonin. Serotonin levels within the synapse drop, then rise again, ultimately leading to downregulation of post-synaptic serotonin receptors (*Goodman and Gilman's pharmacological basis of therapeutics*, 2001).

They have varying degrees of selectivity for the other monoamine transporters, with pure SSRIs having only weak affinity for the noradrenaline and dopamine transporter (Preskorn *et al.*, 2004) . These SSRIs represent a chemically diverse class of agents include: Most common: Fluoxetine, Sertaline, Paroxetine, Fluvoxamine, Citalopram, Escitalopram (Waldinger and Oliver, 2004) and most recently Vilazodone (Khan *et al*., 2011; Mathews *et al.*, 2015). Others: dapoxetine, indalpine (discontinued), zimelidine (discontinued), ericlamine (reached phase III; discontinued in 1999), Panuramine.

The newer antidepressants SSRIs are easy to prescribe and take (usually as a single dose in the morning or at bedtime); cause fewer side effects than some of the older antidepressants, such as the Tricyclic antidepressants (TCAs), and do not require dietary restrictions, such as those required for the mono amino oxidase inhibitors (MAOIs) (Brunton and Parker, 2008).

The antidepressants known as selective serotonin reuptake inhibitors (SSRIs) have become widely used to treat major depression and many other psychiatric disorders

including obsessive compulsive disorder (OCD), panic disorder, generalized anxiety disorder, social anxiety disorder, post-traumatic stress disorder, eating disorders (e.g., bulimia nervosa), and premenstrual dysphoric disorder (Brunton and Parker, 2008).

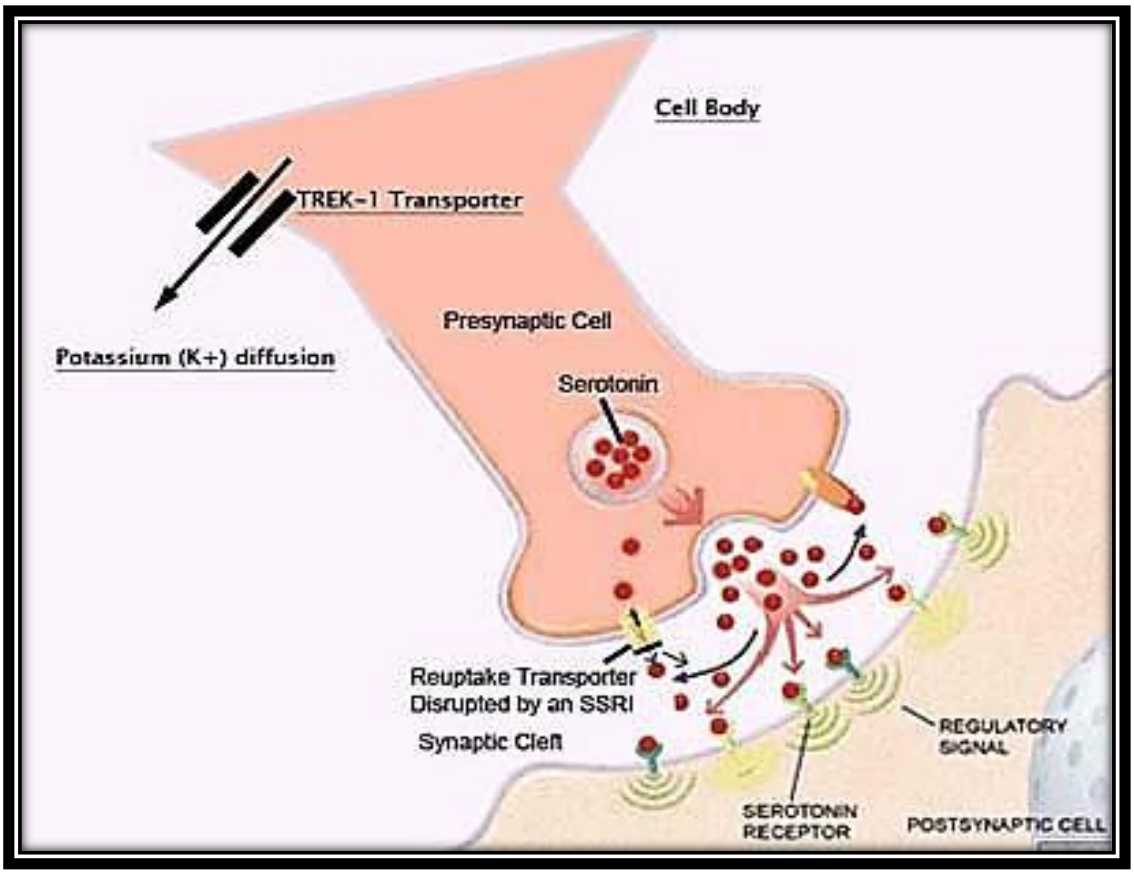

Figure 1.1: Mechanism of action of SSRIs (inhibition of serotonin reuptake to the presynaptic cell). **Kate Donavan (2013). Psychopathology sum-up: Types of antidepressants. ASHILEY F MILLER. The ORBIT; https://the-orbit.net/ashleyfmiller/tag/ssris/**

1.5 Fluoxetine

1.5.1 Fluoxetine discovery

In the early twentieth century, depression was identified as 'melancholia', and it was mainly treated with barbiturates and amphetamines. It was not until the 1950s when the first two compounds with more potent antidepressant activity were developed, named antidepressants. They are iproniazid, the first monoamine oxidase inhibitor (MAOI), and imipramine, the first tricyclic antidepressant (TCA). The emergence of these two antidepressant drugs revolutionized psychiatry and the pharmaceutical industry. Indeed, the discovery of these new treatments for major depressive disorder (MDD) led to the

development of new theories about the pathophysiology of the mood disorder. Ten years later, other TCAs were synthesized (amitriptyline, nortriptyline, desipramine and clomipramine), some of which are still in use to treat depression and other pathologies. In 1965, the monoaminergic hypothesis of depression was postulated (Schildkraut, 1965), which implicated noradrenergic and serotoninergic dysfunction in depression. As a result, some pharmaceutical companies focused their research on the search for new drugs that specifically target 5-HT reuptake. Thus, an SSRI was developed by Eli Lilly and Company, the compound numbered LY110140 (fluoxetine) was initially approved as a drug for medical use in Belgium in 1986, although it was not approved by the FDA until 1987, under the name of Prozac®. Numerous clinical trials reported that the antidepressant efficacy of fluoxetine was as potent as the TCA but with fewer side effects due to its selective profile (Bremner, 1984; Perez-Caballero *et al*., 2014).

Fluoxetine is one of the class termed SSRIs which is a 5-hydroxy tryptamine (5HT) or serotonin agonists, acting to increase the amount of 5-HT within the synaptic cleft. (Breggin and Breggin, 1995).

Fluoxetine is currently one of the most commonly prescribed antidepressant medications for children and adolescents, and its use appears to be increasing internationally (Zito *et al.,* 2002; Murray *et al*., 2004; Aras *et al.,* 2006). It is approved for treatment of depression and obsessive compulsive disorders of both child and adolescents in 2003. Despite this, there has been ongoing speculation regarding safety and efficacy in this younger populations (Garland, 2004; Cohen, 2007).

Whittington *et al.* (2004) said that in view of the evidence for efficacy and no increased risk of serious adverse effects, fluoxetine seems to have a favorable risk–benefit profile for treatment of children and young people than other SSRIs.

1.5.2 Chemical structure and chemical formula

Fluoxetine hydrochloride is a psychotropic drug for oral administration. It is designated (±)–N–methyl–3–phenyl–3–[(α,α,α–trifluoro–ptolyl)oxy] propylamine hydrochloride and has the empirical formula of C17H18F3NO•HCl. Its molecular weight is 345, and its structural formula (Brunton and Parker, 2008) is shown in figure (1.2).

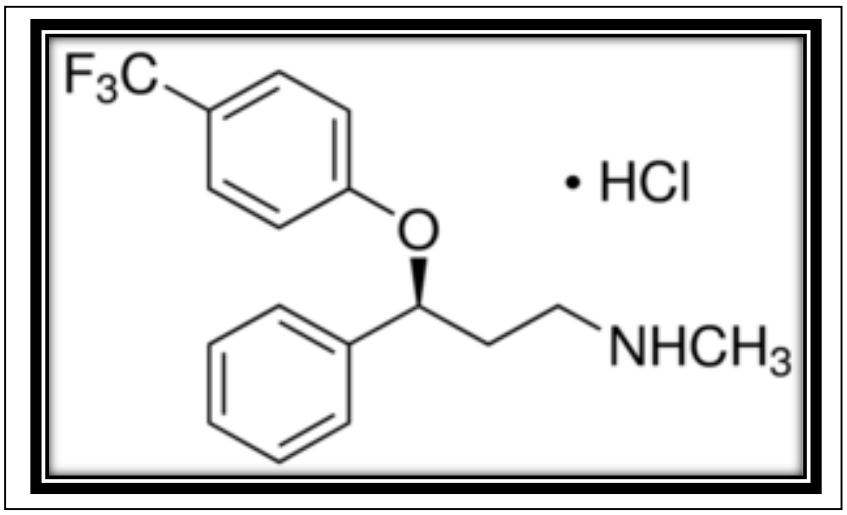

Figure 1.2: Chemical structure of fluoxetine hydrochloride

1.5.3 Biochemistry

Fluoxetine (3-(p-trifluoromethylphenoxy)-Nmethyl-3-phenylpropylamine) is an SSRI that exists as a racemic molecule, with the R (-) and S (+) enantiomers showing equal potency as inhibitors of 5-hydroxytryptamine (5-HT) uptake in both in vitro and in vivo uptake assays (Robertson *et al*.,1988). Moreover, fluoxetine is metabolized by N-demethylation to norfluoxetine, which is an active metabolite which also acts as an SSRI but with a stronger potency than the parental compound (Hyttel *et al*.,1994). This active metabolite also exists in an enantiomeric form, but unlike fluoxetine enantiomers, S-norfluoxetine is over 20-fold more potent in inhibiting 5-HT uptake than the (R)-enantiomer (Wong *et al*., 1993; Perez-Caballero *et al*., 2014).

1.5.4 Mechanism of action

Fluoxetine is a prototype of SSRIs which act on the neurotransmitter serotonin. Serotonin is normally released into the synapse between the nerve cells and is either destroyed or reabsorbed back into the cell that released it. SSRIs block this reuptake causing more serotonin to accumulate in the synapse, thus the concentration of serotonin in the cleft is heightened and neuronal activity is enhanced (Brunton and Parker, 2008), as can be seen in figure (1.3). Specific Transporters are involved in the neuronal reuptake of neurotransmitters and the regulation of their levels in the synaptic cleft as seen in figure (1.1), the serotonin transporter (SERT) is a glycoprotein with 12 trans membrane regions embedded in the axon terminal and cell body membranes of serotonergic neurons. These SERT plays a role in the reuptake and clearance of serotonin in the brain (Joseph *et al.*,

2003).

Membrane transporters are the targets of many drugs, thus SERT is the specific target of the SSRIs (e.g. fluoxetine and paroxetine) and one of several targets of TCAs antidepressants (e.g. amitriptyline), SSRIs allosterically inhibit the SERT transporter by binding the receptor at a site other than active binding site for serotonin. At therapeutic doses, about 80% of the activity of the transporter is inhibited (De Battista, 2009).

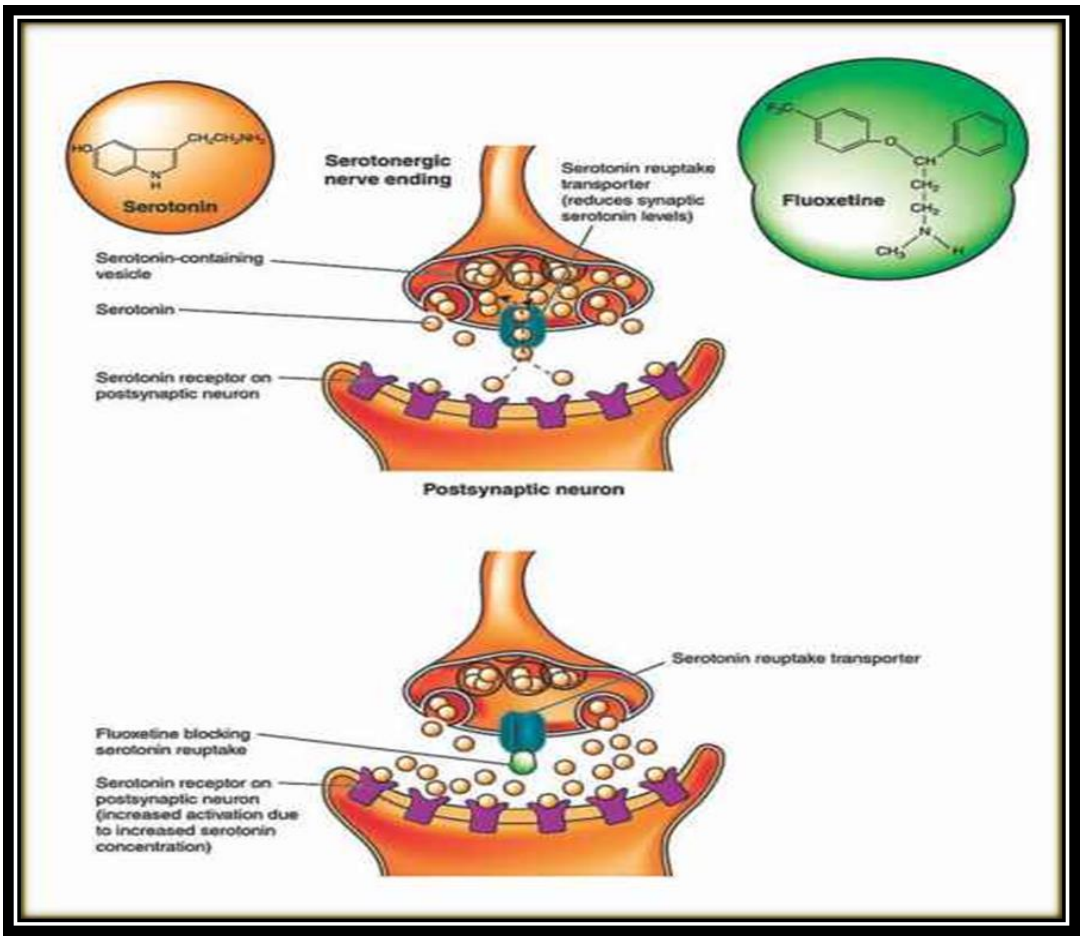

Figure 1.3: Mechanism of action of Fluoxetine.

Drug time (2017). Antidepressants. Fluoxetine. Sertraline. Paroxetine. Citalopram. Fluvoxamine.
https://www.drugtimes.org/antidepressants/fluoxetine-sertraline-paroxetine-citalopram-fluvoxamine.html#

A new theory of the mechanism of action of fluoxetine has been postulated using a unique and relatively simple cell–based fluorescent assay. Berkeley (2011) have identified a means by which fluoxetine, suppresses the activity of the TREK1 potassium channel, which is accompanied by an unbinding of the protein's C–terminal domain from the membrane. This is the first observation of the mechanism by which TREK1 might be regulated by antidepressant drugs. TREK1 activity has been implicated in mood

regulation and could be an important target for fluoxetine and other antidepressant (Berkeley, 2011).

The way through which fluoxetine produced his action in the short and long term therapy (acute and chronic administration) have been discussed in many studies and there is a lot of controversy and there is still a need for more studies to get rid of ambiguity.

Acute fluoxetine administration enhances extracellular 5-HT levels, in conjunction with a decrease in both the synthesis and turnover of 5-HT in the raphe nuclei (Bel and Artigas, 1996) and unlike other SSRI, fluoxetine increases dopamine and noradrenaline concentrations in the prefrontal cortex (Bymaster *et al*., 2002). The increase in 5-HT has been reported in the raphe nuclei and other brain regions, such as the frontal cortex, striatum, diencephalon or hippocampus (Bel and Artigas, 1996). This large increase in 5-HT that have been noted in the raphe nuclei, may negatively control cell firing and 5-HT release into terminal areas including the frontal cortex by the activation of somatodendritic 5-HT1A auto receptors and this may explain why the 5-HT increase in the frontal cortex by acute fluoxetine treatment is smaller than that in the raphe nuclei (Perez-Caballero *et al.*, 2014).

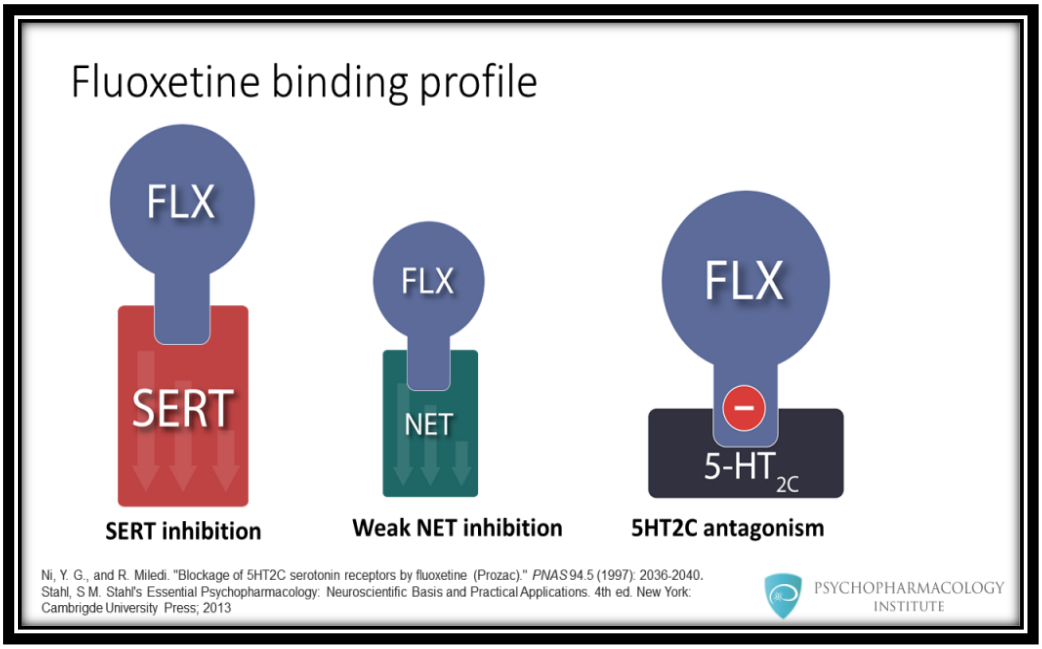

Figure 1.4: Affinity of fluoxetine to different receptors

Stahl S M. Essential psychopharmacology. Neurosientific basis and practical applications. 4th ed. New York. Cambrigde University Press,2013

It has been demonstrated that fluoxetine acts as a 5-HT2C receptor antagonist due to its relative affinity for this receptor as can be notice in figure (1.4) (This receptor subtype exerts inhibitory control on both ventral tegmental dopaminergic and locus coeruleus noradrenergic neurons) (Palvimaki *et al*., 1996), this is because unlike other SSRI, fluoxetine increases dopamine and noradrenaline concentrations in the prefrontal cortex, as measured by micro dialysis.

It was suggested that this effect might reflect an interaction with the 5-HT2C receptor, and indeed, it has been demonstrated that fluoxetine acts as a 5-HT2C receptor antagonist. Thus, the ability of fluoxetine to block 5-HT2C receptor is the most plausible explanation for the cortical increase in catechol amines (Bymaster *et al*., 2002).

Chronic fluoxetine administration induces a persistent increase in 5-HT levels in several brain regions, such as the diencephalon, striatum, hippocampus and frontal cortex, without altering those of cortical noradrenaline and dopamine. Initially, the simple accumulation of higher plasma levels result from chronic fluoxetine administration and its metabolite, caused the sustained 5-HT enhancement due to their long half-life. However, this would appear to be unlikely given that residual drug was still present and the enhanced extracellular 5-HT levels were promptly restored after acute treatment. Thus, several adaptive mechanisms associated with 5-HT neurotransmission have been proposed to explain the persistent changes of extracellular 5-HT after chronic fluoxetine treatment (Perez-Caballero *et al*., 2014).

Hensler in his study in 2002 has been suggested that the desensitization of 5-HT1A auto receptors may be due to alterations in their signal transduction, which involves G-protein. Other studies suggested that fluoxetine negatively regulate the release of 5-HT in terminal areas by desensitization of raphe somatodendritic 5-HT1A auto receptors (Sharp *et al*., 1989; Le Poul *et al*., 2000). Furthermore, electrophysiology and micro dialysis assays demonstrated that the persistent increase in extracellular 5-HT induced by chronic fluoxetine administration might be explained by the desensitization of terminal 5-HT1B auto receptors, whose activation exerts a feedback inhibition of 5-HT release (Blier *et al*., 1988; Newman *et al.*, 2004).

There is some controversy regarding the role of 5-HT transporters in the adaptive changes following chronic fluoxetine administration (Le Poul *et al.*, 2000). For example, Gobbi *et al*. in his study in 1997 said that long-term treatment with fluoxetine does not lead to robust alterations in 5-HT receptors (5-HT2 or 5-HT3). While Vidal *et al.* (2009)

proved that long-term treatment with fluoxetine produced functional desensitization involving the adenylate cyclase system and downregulates the density of 5-HT4 receptors.

Recently, Covington *et al.* (2011) in his study has been described that fluoxetine induces epigenetic modifications that may contribute to the therapeutic action of this antidepressant. In this way, modifications in levels of acetylated histones as well as altering the expression of some microRNAs (miRNAs) in several brain areas have been related to depressive pathology. Thus, chronic fluoxetine treatment is able to reverse some of these changes and interestingly, these miRNAs alterations are also reversed by the non-pharmacological electroconvulsive therapy (O'Connor *et al*., 2013). Even more, other new mechanisms have been proposed for this antidepressant, for example a recent study involves a chromatin remodeling factor in the antidepressant effect of fluoxetine (Oh *et al*., 2013; Perez-Caballero *et al*., 2014).

1.5.5 Pharmacokinetics

Fluoxetine is well absorbed from the gastrointestinal tract, 80–95% absorbed following oral administration. Peak plasma concentrations of fluoxetine from 15–55 ng /ml were observed after 6 to 8 hours following a single oral 40 mg dose. Harvey and Preskorn (2001) reported pharmacokinetics parameters of fluoxetine administration of 20 mg orally.

Fluoxetine may be administered with or without food. The systemic bioavailability of fluoxetine does not appear to be affected by food, although it may inconsequentially delay its absorption (Thrasher, 2010). Maximal cerebral effect reported between 8–10 hours. Fluoxetine has a high lipophilic profile, and it appears to bind strongly to plasma protein, which means it is widely distributed. Thus, high concentrations of fluoxetine and its metabolite norfluoxetine reach the brain (Perez-Caballero *et al.*, 2014). It is highly bound to plasma proteins, mostly albumin. Altamura *et al.*, (1994) found in experimental with animals, that fluoxetine is widely distributed in body tissues with the highest concentration in lung and liver (NTP–CERHR, 2004).

Fluoxetine excreted through urine and feces (Siddiqui, 2011), and it undergoes hepatic metabolism and CYP2D6 isoenzyme convert fluoxetine to pharmacologically active metabolite norfluoxetine by demethylation, which is also serotonin reuptake blocker (Wong *et al*., 2005). Fluoxetine has along $t_{1/2}$ of 1–3 days after a single dose or 2–7 days after repeated administration, while norfluoxetine has a $t_{1/2}$ of approximately 7–

15 days. Thus, fluoxetine can be a good choice for a patient who frequently misses medication doses, but, may be problematic for patient with comorbid medical conditions and on multiple medications (Hansaen, 2004).

The elimination half–life of norfluoxetine is about three times longer than fluoxetine as a result fluoxetine has to be discontinued 4 weeks or longer before MAOI can be administered to mitigate the risk of serotonin syndrome (De Battista, 2009). Because of its long half-life, some doctors switch patients from short agents to fluoxetine to create a more gradual withdrawal experience, although success rates utilizing this technique are unknown (Haddad and Anderson, 2007). Also fluoxetine may be a better agent to use in patients with poor compliance as a long half-life (Adams, 2001).

Fluoxetine is converted metabolically to norfluoxetine and other metabolites (Bergstrom *et al*., 1988), and CYP isozymes play an essential role in the clearance of both fluoxetine and norfluoxetine. Furthermore, both compounds inhibited CYP2D6 isozymes *in vitro* and *in vivo.* The (S)-enantiomers of fluoxetine and norfluoxetine are six times more potent than both (R)-enantiomers, and therefore, both compounds can compete with other drugs for their metabolism by CYP2D6, which would explain their potential to participate in pharmacokinetic drug interactions (Stevens *et al*., 1993; Perez-Caballero *et al.*, 2014).

1.5.6 Determination of fluoxetine concentrations in serum by high performance liquid chromatography HPLC

HPLC is currently the most widely used method of quantitative analysis in the pharmaceutical industry and in pharmaceutical analysis laboratories (Reddy, 2007). Chromatography is an analytical technique based on the separation of molecules due to differences in their structure and/or composition (Simpson, 1976; Pungor, 1995; Moffat *et al.*, 2004).

Clinical analysis of fluoxetine is useful for monitoring patients' blood levels, especially during co administration with other drugs because of the long half-life of fluoxetine and its metabolite which may increases risk of drug-drug interactions. The long half-lives of fluoxetine (2-3 days) and norfluoxetine (7-9 days) can, however, lead to pharmacological interactions with other drugs administered to the patients because plasma levels can remain high even weeks after discontinuation of the therapy (Benfield *et al*., 1986; Risley *et al.*, 1990). Reliable and very sensitive analytical methods are needed for this purpose, because plasma concentrations are usually very low (Raggi *et al*., 1999;

Reddy *et al*., 2007).

Several methods have been published for the determination of fluoxetine in human plasma; most of these are chromatographic methods with UV or fluorescence detection including HPLC assays with liquid-liquid extraction or, more recently, solid-phase extraction (SPE) for the pretreatment of biological samples (Risley *et al*., 1990; Raggi *et al*., 1999; Reddy *et al*., 2007).

In 1999, Raggi and his group developed an HPLC method with fluorescence detection for the determination of fluoxetine and its main metabolite norfluoxetine in human plasma. It seems to be a useful tool for clinical monitoring, because it requires small plasma samples and is highly sensitive and highly selective. It is HPLC method with fluorimetric detection for the determination of fluoxetine and norfluoxetine in human plasma, using a 15 mm x 6 mm i.d., 5 gm ResElut C8 reversed phase column with a 50:50 (v/v) mixture of pH 1.9 tetra methyl ammonium perchlorate and acetonitrile, flow rate 1 mL min -1, as the mobile phase, with fluorimetric detection (230nm, 290 nm).

Many researchers try to developed methods simple, fast, more sensitives, low cost and can be used to assess fluoxetine levels in human plasma in pharmacokinetic studies, in clinical monitoring as well as and in overdose cases such as (Gleiter *et al.*, 1994; Eap *et al.*, 1996; Gevirtz *et al.*, 1999; Kristoffersen, *et al*., 1999; Frahnert *et al*., 2003; Thompson *et al.*, 2004; Vlase *et al*., 2005; Unceta *et al*., 2007). Also Tuchilă *et al.* (2015) which develop HPLC method with fluorescence detection for the quantification of fluoxetine in human plasma. The method was fully validated and all validation parameters are within acceptable limits according to bioanalytical method validation guidelines: the method is linear, accurate and precise on the domain of concentration 0.1-1.0 μg/mL, appropriate to therapeutic plasmatic concentration of fluoxetine (0.05-0.48 μg/mL). This makes the method useful for monitoring the patients under treatment. The method is simple, fast (3 minute time of analysis) and the applicability was checked using plasma samples spiked with fluoxetine, extracted using a solid phase extraction technique. It can be considered that the method developed can be used in pharmacokinetics studies and in therapeutic drug monitoring for patients under treatment with fluoxetine.

1.5.7 Pharmacodynamics profile

1.5.7.1 Inhibition of monoamine uptake: In vitro uptake studies confirmed the strong capacity of fluoxetine to inhibit 5-HT uptake, greater than its affinity for other monoamines (Owens *et al*., 1997). In vivo uptake studies into rat brain synaptosomes also

demonstrated that acute fluoxetine administration produced a significant reduction in 5-HT uptake (57%) compared with controls but not that of noradrenaline or dopamine (Wong *et al*., 1975). The brain regions with the most pronounced reduction in 5-HT uptake were the cerebral cortex and brainstem, whereas fluoxetine administration failed to inhibit uptake into synaptosomes in cerebellum. In vivo studies were carried out to evaluate the duration of the effects of fluoxetine on 5-HT uptake inhibition, demonstrating that maximal inhibition occurred after 4 h and that uptake was restored to normal levels 48 h after administration of fluoxetine. However, throughout this time course, the uptake of noradrenaline was unaltered by fluoxetine administration (Wong *et al.*, 1975). The effect of fluoxetine was long lasting compared with the time course of other antidepressants, which could reflect the extremely long half-life of both fluoxetine and its active metabolite, norfluoxetine. Overall, these data suggest that the metabolite plays an important role for the therapeutic effect of fluoxetine (Perez-Caballero *et al.*, 2014).

1.5.7.2 Transporters and receptors binding: Several competitive binding assays with monoamine transporters showed that fluoxetine presents a strong affinity for the 5-HT transporter and only a weak or no affinity for the noradrenaline and dopamine transporters, respectively (Owens *et al.*, 1997; Bymaster *et al*., 2002; Wood *et al.*, 1986). Therefore, these data confirmed the 5-HT selective profile of this compound as in figure (1.4). Furthermore, fluoxetine showed relatively weak affinity for 5-HT receptors, as measured by radio ligand binding to the 5-HT1 (A, B, C and D), 5-HT2 and 5-HT3 subtypes, although the strongest affinity was found for 5-HT2 receptors (Hyttel, 1994; Owens *et al*., 1997; Koch *et al*., 2002). Additional studies were carried out to evaluate the interaction of fluoxetine with other neurotransmitters receptors, with radio ligand-binding assays showing that fluoxetine has low affinity for D1 and D2 dopaminergic, alfa and beta-adrenergic, muscarinic cholinergic and histamine H1 receptors (Hyttel, 1994; Perez-Caballero *et al.*, 2014).

1.5.8 Indications and usage

Although fluoxetine and SSRIs can immediately change extracellular levels of serotonin in the central nervous system. Therapeutic effects of these drugs usually require weeks of treatments (Stahl, 1998). In this way, to accelerate the clinical action of fluoxetine and even to improve its antidepressant efficacy, preclinical data suggest that

can be used strategies based on fluoxetine treatment in combination with antagonists of the 5-HT desensitized receptors after long-term treatment (Artigas, 1993; Zhang *et al.*, 2000; Vidal *et al.*, 2009).

The pharmacologic action and the clinical indications of fluoxetine has been reviewed in many studies (Wong *et al.*, 1995; Stokes and Holtz, 1997; Grimsley and Jann, 2001).

- **Depression**

Fluoxetine was approved by FDA in December 1987, for the treatment of depression (ICSI, 2004). Fluoxetine has been specifically approved for use in geriatric depression in October 1999, but in February 2001, the FDA approved fluoxetine for maintenance treatment of depression. In January 2003 FDA approved fluoxetine for pediatric use in depression (Cohen, 2002).

Fluoxetine was used to treat depression in a dose equals to 20 mg/day in the morning in adults, may be increased to a maximum of 80 mg per day. Generally, fluoxetine may not the drug of first choice for patients in whom a rapid antidepressant effect is important or for those who are agitated, because of slow onset of action, but it may have advantages over other SSRIs in patients who are poorly complaint with treatment and those who have previously had troublesome discontinuation symptoms (Hansaen, 2004).

Earlier investigations into the efficacy of fluoxetine for the treatment of pediatric and adolescent depression showed limited success (Rowse, 2010). They showed similar rates of improvement across the treatment and placebo groups (Andersen and Navalta, 2004). In contrast, some studies (Whittington *et al.*, 2004; March *et al.*, 2004) concluded that fluoxetine has a favorable risk–benefit profile.

Many studies showed that fluoxetine have has similar efficacy in treatment of depression with paroxetine, citalopram and sertaline, with comparable side effects including nausea and vomiting (Adams, 2001).

Fluoxetine is the only SSRIs approved for the treatment of depression in children 8–18 years (ICSI, 2004; Kaiser, 2005; American Psychiatric Association, 2000) and the recommended dose was: 10–20 mg/day; lower–weight children can be started at 10 mg/day.

- **Major anxiety disorders**

Fluoxetine was used to treat Major anxiety disorders including post-traumatic stress disorder (PTSD), obsessive–compulsive disorder (OCD), social anxiety disorder, generalized anxiety disorder (GAD), and panic disorder and get FDA–approval in July 2002 (Cohen, 2002).

Fluoxetine was approved by FDA for the treatment of OCD in adults in Febuary1994 (Cohen, 2002). SSRIs are considered first–line treatment for PTSD and can benefit a number of symptoms including anxious thoughts and hyper vigilance. The recommended dose in PTSD is 20–40 mg/day (De Battista, 2009).

Fluoxetine is used to treat OCD in a dose of 20–60 mg/day (adolescents and higher weight children, in7–17 years: 10 mg/day; may increase to 20 mg/day) (Bandelow *et al*., 2008).

- **Premenstrual dysphoric disorder (PMDD)**

Fluoxetine received approval for treatment of PMDD in July 2000 (Cohen, 2002). Approximately 5% of women in the child–bearing years will have prominent mood and physical symptoms during the late luteal phase of almost every cycle. The SSRIs are known to be beneficial to many women with PMDD, and fluoxetine and sertraline have been approved for this indication (De Battista, 2009). Fluoxetine used to treat PMDD in a dose of 20 mg/day continuously, or 20 mg/day starting 14 days prior to menstruation and through first full day of menses (repeat with each cycle).

The mechanism of action of fluoxetine in PMDD is unknown, but is presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. Studies at clinically relevant doses in humans have demonstrated that fluoxetine blocks the uptake of serotonin into human platelets. Studies in animals also suggest that fluoxetine is a much more potent uptake inhibitor of serotonin than of norepinephrine (Medication Guide FDA, 2009).

- **Eating disorders**

In November 1996, the FDA granted fluoxetine approval for the treatment of bulimia nervosa in adults, it was the first drug to be approved for this condition, the dose recommended in eating disorders was; 60 mg/day (Cohen, 2002). Bulimia nervosa and anorexia nervosa are potentially devastating disorders. Anorexia is often chronic and may be fatal in 10% or more cases (Potter and Hollister, 2007).

- **Premature ejaculation**

Antidepressants are commonly associated with inducing sexual adverse effects, some of these effects may prove useful for some sexual disorders. For example, SSRIs are known to delay orgasm in some patients, for this reason, it is sometimes used to treat premature ejaculation (De Battista, 2009).

1.5.9 Adverse reactions

Although the selective serotonin reuptake inhibitors (SSRIs) were used as a first line treatment for depression, they were not devoid of side effects. Most short-term treatment-related side effects of SSRIs are transient and disappear after a few days or weeks. However, following long-term treatment with the SSRIs some adverse events may occur. They are often difficult to recognize since they often resemble residual symptoms of the depression (Moret and Isaac, 2007). The most troubling adverse events seen during long-term SSRI therapy are Sexual dysfunction, weight gain and sleep disturbance (Ferguson, 2001; Moret and Isaac, 2007).

Since, SSRIs apparent pharmacological activity is the inhibition of the reuptake of serotonin. Most of side effects result from an over-stimulation of various serotonin receptors in both the brain and the periphery (Lieberman, 2003). The most common side effects associated with SSRIs such as nausea and headache, nervousness, insomnia and sexual dysfunction (Kelsey, 2001), all this symptom are related to the stimulation of 5-HT2 and 5-HT3 receptors.

Many of the side effects of SSRIs are transient and subside over time, and can be minimized by having patients take the drug with meals and starting treatment with low doses followed by a slow titration to recommended doses (Kelsey, 2001).

Fluoxetine adverse effect like other SSRIs is mostly dose dependent, appear in up to 75% of patients on normal doses (Kauffman, 2009). It appears similar in children to those in adults, consisting most commonly in headache, nausea, diarrhea, insomnia, nervousness, anxiety and somnolence (Lilly Sarafem Labeling, 2002; Lilly Prozac labeling, 2003).

1.5.9.1 General adverse reactions

- **Gastrointestinal adverse effects**

Nausea (occur in 21% as recorded incidence in many studies), anorexia (9%), diarrhea, dry mouth, insomnia (15% and it sufficient to result in stopping the medication), dyspepsia (6%), stomatitis and upper gastrointestinal bleeding (reviewed suggestion decreased platelets aggregation in response to serotonin reuptake inhibition (NTP–CERHR, 2004; ICSI, 2004).

Jing Zhao (2010) found that fluoxetine caused nausea more than paroxetine, but less than sertaline and venflaxine. Also he found that diarrhea incidence was higher with fluoxetine, rather than paroxetine and venflaxine.

- **Nervous system adverse effects**

Headache, nervousness, insomnia, drowsiness, tremor, akathisia, fatigue, sleep abnormalities, dyskinesia, worsening of Parkinson disease, and cognitive dysfunction, extrapyramidal symptoms (EPS). All are adverse effects have been reported in FDA patient information sheet (2006).

A variety of EPS have been reported to be associated with the SSRIs, ranging from tremor to dystonic reactions (Goldberg, 1998). Meltzer *et al*. (1989) reported a severe dystonic reaction in a 25–year old man on the fourth day of treatment with 30mg of fluoxetine twice daily. While Coulter and Pillans (1995), reported on the prevalence of EPS. There are 15 reports of EPS among 5555 patients receiving fluoxetine.

Akathisia has been reported to occur in the first week of treatment with SSRIs and may occur with the first dose. Although akathisia has been reported in association with fluoxetine and sertraline, no cases have been reported with paroxetine (Goldberg, 1998).

Lipinski *et al*. (1989) reported their estimation of incidence between 10% and 25%. While Hamelton and Opler (1992) suggested that the mechanism of fluoxetine induced akathisia would be via serotonin–induced inhibition of the dopaminergic neurons.

Jing Zhao (2010) found that fluoxetine caused headache and insomnia more than venflaxine, but less than paroxetine and sertraline.

- **Psychiatric adverse effects**

It has been reported suicidal ideation, mania, hypomania, agitation and depersonalization syndrome. In May 1990, the U.S. FDA required the manufacturer of

prozac, Eli Lilly and Company, to add "suicidal ideation "and "violent behaviors" to the post introduction reports sections of its label (Breggin, 2003).

Mania and psychosis is the extreme end of a stimulant continuum that often begins with lesser degrees of insomnia, nervousness, anxiety, hyperactivity and then progress toward more severe agitation, aggression, and varying degrees of mania (Breggin, 1992). Mania has been reported with fluoxetine, but occurs with low incidence (about 1%) and less than TCA (Goldstein and Goodnick, 1998).

- **Genitourinary adverse effects**

Sexual dysfunction includes male and female anorgasmia, decreased libido, ejaculatory dysfunction and impotence. Increasing serotonergic tone at the level of the spinal cord and above is associated with diminished sexual function and interest. As a result, at least 30–40% of patients treated with SSRIs report loss of libido and delayed orgasm. The sexual effects often persist as long as the patient remains on the antidepressant but may diminish with time (De Battista, 2009).

In experimental animals, altered estrous behavior, altered sexual receptivity and reduced sexual motivation were observed (NTP–CERHR, 2004).

- **Hematologic adverse effects**

Fluoxetine interfere with platelets function may cause petechiae, increased bleeding time and gastrointestinal hemorrhage (FDA Guide, 2009). More than 99% of whole body serotonin is stored in platelet and under normal circumstances platelets release serotonin at the site of vascular tears, leading to platelets aggregation and vasodilatation (allowing for clotting without thrombosis). Since fluoxetine as SSRIs block serotonin uptake into platelets, it may be expected to potentially impair aggregation and increase bleeding time (Celada *et al*., 1992). Aranth and Lindberg (1992) reported a case of a 40-year-old woman receiving 60 mg/d of fluoxetine who developed heavy menstrual flow, spontaneous ecchymosis, and splenomegaly, which led to discontinuation of fluoxetine.

The ecchymosis faded and the spleen size decreased about 4 days after discontinuation fluoxetine treatment. Fisher et al. (1995) have reported on the incidence of adverse effects in patients receiving either sertraline or fluoxetine. Four patients reported bleeding as an adverse effect from a total 2786 patients.

- **Endocrine adverse effects**

Fluoxetine causes syndrome of inappropriate antidiuretic hormone (SIADH) particularly in elderly patients (Medication Guide, 2009). The SIADH is characterized by a reduced ability to excrete water, resulting in extra-cellular dilution with resulting hyponatremia.

Cases of SIADH associated with SSRIs have been described in conjunction with fluoxetine, paroxetine, or sertraline treatment. It is not known how the SSRIs induce SIADH. It has been suggested that the SSRIs cause the release of ADH. Another possible explanation is that the SSRIs increase renal responsiveness to ADH (Goldberg, 1998).

- **Other adverse effects**

Fluoxetine does not cause weight gain that may occur with TCA (De Battista, 2009). Fluoxetine is more likely to produce appetite suppression and weight loss is reviewed by Goldstein and Goodnick (1998) leading to off label use of this medication in obesity, which also have been reviewed by Stokes and Holtz (1997). Weight loss due to the anorexic effects of fluoxetine, have been demonstrated in a study of 20 non depressed obese women receiving 60 mg fluoxetine a day, indicated that fluoxetine increased resting energy expenditure and basal body temperature which can limit food consumption (Lilly Prozac Labeling, 2003; Medication Guide, 2009). Rash (4%), pruritus (2%) and other allergic reaction have been reported with fluoxetine (Lilly Annual Report 2001; Clinical pharmacology and biopharmaceutics, 2002).

1.5.9.2 Oral adverse reactions

The oral side effects which have been reported may include xerostomia (affecting approximately 18% of patients), dysgeusia (altered taste sensations), stomatitis and glossitis.

Fluoxetine and SSRIs produce no significant changes in salivation (Hunter and Wilson, 1995a) but dry mouth may still be seen (Ellingrod and Perry, 1994; Ravindran *et al.*, 1997; Trindade *et al.*, 1998). Patients receiving fluoxetine may develop a movement disorder that includes clenching, grinding of the teeth (bruxism) or both, further worsening the periodontal condition. This may occur, because these medication increases extra pyramidal levels of serotonin, thereby inhibiting dopaminergic pathways that control movements (Friedlander, 2001; Guggenhiemer, 2003).

1.6 Xerostomia

Xerostomia is defined as a subjective complaint of dry mouth that may result from a decrease in the production of saliva (Sreebny, 1989; Navazesh *et al*., 1992; Thomson *et al*., 2001; Guggenheimer *et al.,* 2003). Studies have found the condition in17–29% of sampled populations based on self–reports or measurements of salivary flow rates (Sreebny and Valdini, 1987; Nederfors *et al.*, 1997). Complaints of dry mouth generally are more prevalent in women (Neville *et al*., 2002; Guggenhiemer, 2003).

Xerostomia generally accompanied by salivary gland hypo function and adverse reduction in the secretion of unstimulated whole saliva (Sreebny, 1989), but it is not necessarily reflected in the actually measured flow rate (Nederfors *et al.,* 1997).

A visual analogue scale (VAS) can be used to assess the severity at the initial visit and to evaluate the patient's response to the recommended therapy at subsequent visits. VAS is commonly used in the assessment of pain, but also can be used to assess salivary-related complaints. Dentists or designated staff members ask patients a series of questions and instruct them to mark their responses to each question by placing vertical lines on a 100-millimeter horizontal scale. The scale is labeled at both ends. One end represents the maximum intensity or frequency of the presenting condition, and the other end represents the absence of the condition. For example, if a patient is asked to rate dryness of the mouth, the scale is labeled "not dry at all" at one end and "very dry" at the other end. The practitioner then compares future scores with this baseline score to determine if the patient's condition is improving or worsening (Navazesh, 2003). This questionnaire has previously been tested for validity and reproducibility and was found to have high test–retest correlations, high internal consistency, and sensitivity for changes in dryness (Wewers and Lowe, 1990; Eisbruch *et al*., 2001; Jabbari *et al*., 2005; Meirovitz *et al.*, 2006).

1.7 Saliva

Saliva represents an increasingly useful auxiliary means of diagnosis. Sialometry and sialochemistry are used to diagnose systemic illnesses, monitoring general health, and as an indicator of risk for diseases creating a close relation between oral and systemic health.

The primary constituents of saliva are water, proteins and electrolytes. These components enhance taste, speech, and swallowing and facilitate irrigation, lubrication,

and protection of the mucous membranes in the upper digestive tract. Additional physiological functions of saliva provide antimicrobial and buffering activities that protect the teeth from dental caries (Guggenheimer, 2003).

The combined secretions from the various salivary glands are termed "whole saliva". Whole saliva contains components in addition to salivary secretions including gingival crevicular fluid, leukocytes, epithelial cells and microorganisms, as well as, possibly, food debris, blood and viruses.

The amount of saliva in the mouth is not constant and varies within a person over time and between individuals (Ship *et al.,* 1991).

1.7.1 Salivary flow rate

At rest, without exogenous or pharmacological stimulation, there is a small, continuous salivary flow denominated basal unstimulated secretion, present in the form of a film that covers, moisturizes, and lubricates the oral tissues. Whereas, stimulated saliva is produced in the face of some mechanical, gustatory, olfactory, or pharmacological stimulus, contributing to around 80%-90% of daily salivary production (Edgar, 1992; Axelsson, 2000). The salivary flow is categorized as unstimulated or resting (Guggenheimer, 2003) on which, the parotid, submandibular, sublingual and minor mucous glands contribute about 25%, 60%, 7–8%, and 7–8% respectively to whole saliva and to stimulated on which the parotid glands contribution increases by at least 10% (Dawes, 2008).

The salivary flow rate is influenced by a large number of factors, including the degree of hydration, body position, exposure to light, previous stimulation, circadian and circannual rhythm, gland size and drug use (Edgar *et al*., 2004; Dawes, 2008).

Daily salivary output is estimated to be approximately one liter per day, and flow rate fluctuated by as much as 50 percent with diurnal rhythms. The unstimulated flow rate average 0.3–0.4ml per minute, but the range is wide (Enberg *et al*., 2001; Dawes, 2008).

Salivary gland secretion is mainly under autonomic nervous control, Parasympathetic stimulation produces copious saliva of low protein concentration, while sympathetic stimulation produces little saliva, but of high protein concentration and may thus give a sensation of dryness (Carlson, 2000).

The salivary flow index (SF) is a parameter allowing stimulated and unstimulated saliva flow to be classified as normal, low, or very low (hypo salivation) (Tenovue *et al.,*1994; Malamud, 2006). In adults, normal total stimulated SF ranges from (1–3) ml/

min, low ranges from (0.7–1.0) ml /min, while hypo salivation is characterized by a SF of less than (0.7) ml /min.

The normal unstimulated SF ranges from 0.25–0.35 ml/min, low ranges from (0.1–0.25) ml /min, while hypo salivation is characterized by a SF of less than (0.1) ml/min (Axelsson, 2000; Malamud, 2006). However, the values denominated "normal" for stimulated and unstimulated SF exhibit a large biological variation (Edgar *et al.,* 2004).

It has become apparent that many systemic diseases affect salivary gland function and salivary composition. Studies of the effects of systemic diseases on salivary variables have been valuable in understanding the pathogenesis of the diseases, but their use as diagnostic markers have been limited (Edgar *et al.,* 2004).

1.7.2 Salivary α -amylase (SAA)

It is a major secretory protein found in saliva and aids in the initial digestion of starch (Turner and Sugiya, 2002). This enzyme is considered to be a good indicator of properly functioning salivary glands (Enberg *et al.*, 2001). The greater part of this enzyme (80%) is synthesized in the parotids and the remainder in the submandibular glands. SAA is an enzyme that is produced by the acinar cells of the salivary gland. SAA levels follow a diurnal pattern, with low levels in the morning and a steady increase in levels throughout the day. Its release is regulated by autonomic innervation (Turner and Sugiya, 2002; Granger *et al.*, 2007).

SAA were shown to be flow-rate independent (Rohleder *et al*., 2006; Wolf *et al*., 2008). Chatterton and colleagues (Chatterton *et al.*, 1996, 1997) linked levels of SAA to sympathetic activation during physically and psychologically stressful conditions which found to be closely associated with changes in norepinephrine concentrations. Psychological stress activates both the HPA axis, as well as the SAM axis, which manifests as changes in cortisol and SAA output. For example, SAA has been found to respond to psychological stress (Bosch *et al*., 1996; Skosnik *et al*., 2000; Nater *et al*., 2005, 2006; Rohleder *et al*., 2006). Sympathetic stimulation causes high SAA release from the parotid and submandibular acinar cells, whereas parasympathetic stimulation causes low SAA release from the sublingual acinar cells (Kelly *et al.,* 2010).

SAA measurement presents a noninvasive and a highly reliable indicator of SAM activity and also used as a biomarker for stress, as shown in many studies which showed a marked increase in SAA concentration in response to stressful tasks of procedures, such as (Busch *et al*., 1996; 1998; Chatterton *et al.*, 1996; Skosnik *et al*., 2000; Nater *et al*.,

2004; Labudda *et al.,* 2007). Changes in SAA is thought to have implications for health. Two studies by Granger *et al.* (2006; 2007a) who suggested a link between SAA and disease. Levels of SAA also rise in temperature extremes, and academic examinations response to stressful conditions, including exercise (Chatterton, 1996; 1997; Skosnik *et al.,* 2000).

1.7.3 Salivary sodium (Na^+) and potassium (K^+)

These two ions are very important for different physiological activities. Na^+ is the major extracellular cation and plays a central role in the maintenance of normal distribution of water and osmotic pressure. While K^+ is the major intracellular cation (Burtis and Ashwood, 1994).

Na^+, K^+ with chloride in saliva are the most important ions for maintaining the ionic strength of saliva. Studies on the structural element of enamel revealed that Na^+ is present in the enamel of human teeth, in an increasing concentration gradient from the surface to the dentin (Thylstrup and Fejerskov, 1996). The Na^+ and K^+ concentrations of saliva are markedly affected by corticosteroids, especially aldosterone. The Na^+ / K^+ ratio of stimulated whole saliva can be used in diagnosing and monitoring Cushing's syndrome and Addison's disease. Investigators have also demonstrated the diagnostic value of Na^+ / K^+ ratio in primary aldosteronism (Wotman *et al*., 1969).

1.8 Saliva and oral health

Saliva is one of the most important factors in regulating oral health (Katie *et al.*, 2008), with flow rate and composition changing throughout development and during disease and treatment with different type of medications (Dodds and Johnson, 2005). Salivary fluid is an exocrine secretion (Edgar, 1992; Humphrey and Willimson, 2001) consisting of approximately 99% water, containing a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins represented by enzymes, immunoglobulins and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides of importance to oral health.

Saliva is critical for preserving and maintaining the health of oral tissues and has been used as a source of non-invasive investigation of metabolism and the elimination of many drugs. However, it receives little attention until its quantity diminishes or its quality becomes altered (Katie *et al*., 2008.)

Many researchers have made use of sialometry and sialochemistry to diagnose systemic illnesses, monitoring general health, and as an indicator of risk for diseases creating a close relation between oral and systemic health. With advances in microbiology, immunology and biochemistry, salivary testing in clinical and research settings is rapidly proving to be a practical and reliable means of recognizing oral signs of systemic illness and exposure to risk factors. The components of saliva act as a "mirror of the body 's health", and the widespread use and growing acceptability of saliva as a diagnostic tool is helping individuals, researchers, health care professionals and community health programs to better detect and to monitor diseases and to improve the general health of the public (Dodds and Johnson, 2005). Many studies showed that analysis of saliva sample is a convenient means for assessment of physiological conditions, evaluation the serum concentration of medicine and assessment of the severity of an illness (Loewit *et al.,* 1996; Tadasi, 2002).

Saliva is a good indicator of the plasma levels of various substances such as hormones and drugs and can therefore be used as a non-invasive method for monitoring plasma concentrations of medicines or other substances (Dodds and Johnson, 2005).

1.9 Oral health as an integral and critical part of general health of a psychiatry

Drug use is associated with significant detrimental, psychological, nutritional, and social changes, any of which can affect the general and oral health (Guzeldemir *et al.*, 2009). Although oral health problems are rarely serious, they may have significant social, economic and psychological consequences for patients (Nikias, 1985).

Saliva is one of the most important factors in regulating oral health, with flow rate and composition changing throughout development and during disease and treatment with different type of medications like SSRIs. In view of the shortage of data, health care must be judged increasingly on "how well it keeps people healthy" as opposed to "how well it cures diseases (Jones *et al.,* 2006). This concept includes oral health care, in which maintenance of optimal functioning and well-being is an important goal (Guzeldemir *et al.,* 2009).

Although oral health problems are rarely serious, they may have significant social, economic and psychological consequences for patients, including quality of life. It is important, however, to distinguish between clinical oral conditions and patients' perceptions of how oral conditions affect functioning and wellbeing patients. The effect

of oral conditions on physical health may vary with age, sociodemographic and psychological status, and history of significant illnesses (Guzeldemir, 2009).

More knowledge is needed regarding what works to prevent oral health problems and reduce disparities in oral health status experienced by disadvantaged groups. For those with mental illness, the illness-and xerostomia-associated pharmacological management, puts individuals at greater risk for tooth decay, periodontal diseases, and increased requirements for periodontal treatment, dental restorations, and dental execrations (Allomania, 2009).

A volition in individuals with severe mental illness may affect their ability and desire to perform preventive oral hygiene procedures (Friedlander and Mohler, 2001). Furthermore, increased the use of candy, chewing gum, and carbonated beverages to combat xerostomia can further promote tooth decay (Allomania, 2009).

2. AIMS OF STUDY

2. AIMS OF STUDY

2.1 General objectives and benefit of the study

It's very difficult to describe the situation in IRAQ. Some American which are not veteran and visit IRAQ for different purposes said that "War was followed them home". I thought when read that "What about peoples, war is their only home!".

In Iraq since 2003 there is no accurate statistics in all fields especially in health sector which has been affected thoroughly by the war and the number of subjects need psychological health care has been increased due to the increased stress, violence due to the post war conflicts. For these reasons it is necessary to find simple and economical method to detect subjects under stress to help in the prevention of deterioration of their mental state and progress to a more serious condition. This will be the prime objective of this study.

In view of the shortage of data in IRAQ especially from 2003 and with increased susceptible of people to violence and stress and increase susceptibility to psychiatric disorders and increase need for drug treatments, this study was to record type of psychiatric disorders in sample of different patients as an example that may reflect the population, adverse effects of most widely used fluoxetine therapy in our population as a goal to reach maintenance of optimal functioning and well-being and as try to achieve "keep people healthy" as opposed to "how well it cures diseases and the consequence of adverse drug therapy", as a part, encourage the patients to concern with oral hygiene and maintain their oral health as a part of the general health and to report any dental problems including adverse effects of drugs to the psychiatrist.

Drug use is associated with significant detrimental, psychological, nutritional, and social changes, any of which can affect the general and oral health. Fluoxetine was the first SSRIs approved to treat depression in humans and is one of the most widely prescribed antidepressant drugs. Many general and oral adverse effects have been reported with fluoxetine therapy. Some of its observed secondary effects are related to an alteration in the salivary secretion and composition. Despite that fluoxetine was approved by the United States Food and Drug Administration's (FDA) in 1987 for the treatment of depression and then for the treatment of a number of psychiatric disorders in adults and children, it was used in the psychiatric treatment in IRAQ in the ultimate years and there is shortage of data that record efficacy, adverse effects and other about drug due to the non-recognition of the psychiatric disorders and mainly depression by the majority of the

society. Most of the people in the Middle East does not accept the concept of (pharmacological treatment) for the psychiatric disorders especially depression because of some religious believes and social aspects. This study was the first pharmacological study done in Mosul and Iraq on the psychological patients to see the effects of the drug (fluoxetine) on the patients and encourage both the psychiatrist and the patients to detects the adverse effects of the drug and try to avoid it as possible.

Salivary biomarkers are important field of research because of easy, non-invasive and rapid collection of samples compared to the blood and urine samples thereby increasing the patient compliance. There is increased need of biological stress markers to provide an objective, reliable and authentic evidence of stress such as salivary alpha amylase. In this we try to evaluate the usefulness of SAA as a biomarker of stress and if there is a relation between salivary SAA and sodium and potassium levels.

2.2 Specific objectives of the study

The specific aims of the study were to investigate:

1. The usefulness of salivary alpha amylase (SAA) as a good biological marker of stress in psychological patients and check the possibility to use it to detect person on stress like a psychological patient.
2. Salivary sodium and potassium, as a markers of stress. And check the possibility to use them to detect person on stress in conjunction to SAA to detect patients.
3. The biochemical changes in saliva including sodium, potassium and salivary alpha amylase concentrations, and changes in salivary flow rate associated with fluoxetine therapy in psychiatric patients.
4. The effects of psychiatric diseases on the composition of saliva (salivary alpha amylase, sodium and potassium) and salivary flow rate.
5. Objective and subjective measurement of xerostomia and determination of its grades as a result of disease process and fluoxetine therapy.
6. The adverse effects associated with fluoxetine therapy in our population including both general adverse effects on the body at whole and oral adverse effects and their relation with dose and duration of therapy.

7. Fluoxetine concentrations in serum by high performance liquid chromatography (HPLC) and their relation to SAA, Na^+ and K^+ concentrations and adverse effects due to administration of fluoxetine.

3. MATERIALS AND METHOD

3. MATERIALS AND METHOD

3.1 Subject groups

The study carried out on total number of (80) subjects with an average age between (15-59) years.

This case control study was conducted at the psychiatric departments in Ibn Sina and AL Salam Teaching Hospitals in Mosul, and in some private clinic in Mosul during 2014.

Permission to examine the subjects and to perform the research were obtained from the concerned authorities in the Directorate of General Health in Ninava and ministry of health/IRAQ.

The study was done in two major groups: Study group (patients), and Control healthy subjects.

3.1.1 The study group (patients)

Patients were eligible to participate in the study, if they had been diagnosed by their doctors, with a different psychiatric disorders that required treatment with fluoxetine.

The study group was divided into two subgroups according to the duration of fluoxetine therapy.

A. Acute (follow up) group: included patients who had been diagnosed by the psychiatric doctors and prescribed to have fluoxetine therapy for the first time. The design of the study was an 8-weeks open trial. All patients had been provided with fluoxetine capsules starting by (20mg/ day) dose. Then increasing the dose according to their clinical response from (20-60 mg/day). This group consisted of a total of (39) patients (23 M, 16 F), their average age was (35.4±10.7) years.

Three readings (biochemical and clinical parameters) taking from the patients were recorded for this group. The first pre-treatment parameters before they started the administration of fluoxetine, considered as a base line to be

B. Chronic group: Included patients were already on fluoxetine therapy for different doses and durations (more than 8 weeks of therapy).

In this group, (21) patients (7 M, 14 F) accepted to participate in this study, their average age was (33.95) years.

One reading (biochemical and clinical parameters) taking from the patients were recorded for this group.

3.1.2 Control group

This group included (20) nonsmoker healthy subjects (10 M, 10 F) with an average age (35.6) range (17–59years). None of these subjects had a history of any systemic diseases, and the females were not pregnant or lactating.

3.2 Subjects groups assessments

A standard questionnaire form was used containing detailed information about each patient (appendix 1). It included the following information:

I- Case history: Name, age, sex, address, marital state.

II- Medical history: The information was taken from their medical records in the psychiatric department in the hospital which include the diagnosis, family history, treatments (drug types, doses, duration of treatment and side effects) and the past medical or surgical history.

III- Visual analog scale (VAS) for the determination of the grade and severity of xerostomia.

For chronic and control groups screening by medical history, physical examination and sample collection (saliva and blood) was done at one visit, while the screening for follow-up group was done three times, the 1st assessment at base line (pretreatment) visit before the first dose of the fluoxetine, the 2nd assessment was done at (4 weeks) during treatment and 3rd data were collected at the end of trial period (8 weeks).

3.2.1 Evaluation and assessment of xerostomia

Oral dryness feeling or xerostomia is a subjective sensation, and does not reflect a dry mouth in up to one third of cases. It is associated with an unpleasant feeling in the mouth and throat (Nederfors, 2000). In this study the evaluation of xerostomia was done by two methods which are:

3.2.1.1 Visual analogue scale (subjective assessments)

As xerostomia is primarily a symptom, patient self-reporting may be meaningful in assessing its severity. For this reason, a type of xerostomia questionnaire (XQ) was used which called as the visual analogue scale (VAS). The patient self-reported XQ instrument is detailed in table (3.1).

Table (3.1): Xerostomia self-reported questionnaire (XQ)

No	Questions	Score (0-10)
1.	Rate your difficulty in talking due to dryness	
2.	Rate your difficulty in chewing due to dryness	
3.	Rate your difficulty in swallowing solid food due to dryness	
4.	Rate the frequency of your sleeping problems due to dryness	
5.	Rate your mouth or throat dryness when eating food	
6.	Rate your mouth or throat dryness while not eating	
7.	Rate the frequency of sipping liquids to aid swallowing food	
8.	Rate the frequency of sipping liquids for oral comfort when not eating	
	Total scores	

*(Navazesh, 2003; Jabbari *et al*., 2005; Meirovitz *et al.*, 2006).

The questions are equally divided into four items asking about dryness while eating or chewing (2, 3, 5, y 7), and four items about dryness while not eating (1, 4, 6 y 8). Subjects rated each symptom on scale from 0 to 10, with higher scores indicating greater dryness or discomfort because of dryness. Each item score was added, and the sum was transformed linearly to produce the final summary score, with higher scores denotes worse xerostomia (Wewers and Lowe, 1990; Eisbruch *et al*., 2001; Jabbari *et al*., 2005; Meirovitz *et al.*, 2006).

3.2.1.2 Salivary flow rate method (objective Assessment):

This is usually done after the collection of each saliva samples. The salivary flow rate (FR) was calculated as the volume in (ml) of the sample collected divided by the time in (minute) required for collection.

$$\textbf{Salivary flow rate } (\textbf{ml/min}) = \frac{\textbf{Salivary volume}(\textbf{ml})}{\textbf{Time}(\textbf{min})}$$

The grade of xerostomia was divided according to the amount of saliva produced in one minute as shown in Table (3.2).

Table (3.2): Objective grading system for xerostomia

Grade	Salivary flow rate (ml/min)
1	Flow rate ≥ 0.2 ml/min
2	Flow rate 0.1–0.2 ml/min
3	Flow rate ≤ 0.1 ml/min

*Whole-mouth, un stimulated flow rates. ** Navazesh, 2003; Nederfors, 2000

3.2.2 Safety and tolerability assessment

Assessment of drug safety was carried out during the treatment period by recording the incidence and intensity of adverse effects reported by the psychiatrist in the patient record.

Safety and tolerability were assessed at the baseline (pre-treatment) visit, fourth week during treatment and at the end of trial period (8 weeks) or at the time of withdrawal, the cause and the time of withdrawal were also recorded.

3.3 Methods

3.3.1 Collection of saliva samples

Whole unstimulated saliva samples were collected with the subject setting quietly using spitting method for 5 minutes (Dittmer, 1991; Kashmoola, 2000; Khudir, 2008).

The samples were usually collected about 2 hours after breakfast, usually between (9 to 12 pm). The subject was asked to rinse his mouth many times with water to remove all food debris from the mouth. Sterile graduated plane tube and a glass funnel was given to each subject for spitting the collected saliva. The volume of the saliva sample collected was recorded. Top of the tubes were closed to prevent any contamination of salivary samples. The sample were kept cold in ice container and freeze (-20ºC) within 30 min of collection because many analytes are usually not stable at room temperature (Navazesh, 1993; Nater *et al*., 2005).

The salivary sample treated on the day when the samples to be assayed by complete thawing of the samples, vortex, and then centrifuge for 15 minutes at approximately 3.000 RPM (1500 x g). Freezing saliva samples will precipitate the

mucins, which can make accurate pipetting difficult (Navazesh, 1993; Nater *et al*., 2005; Khudir, 2008). Assays were performed using only clear saliva, avoiding any sediment present in the bottom of the tube. Tubes were re-centrifuged following each freeze-thaw cycle as additional precipitates that may be developed upon re freezing.

3.3.2 Blood samples

Patients' blood samples were drawn in red-top Vacutainer blood-collection tubes (16 x 100 mm, 10-mL, no. 6530; Becton Dickinson, Rutherford, NJ). Specimens were allowed to clot and then centrifuged. Serum was removed from cells, aliquoted, and stored at -20 until analysis.

3.4 Materials

3.4.1 Chemical materials and kits

The general laboratory chemicals used in this study were of analaR-grades. In addition, standard kit was used to measure SAA parameter suggested in this study. Tests were performed and interpreted following instructions outlined in each kit. Specific chemicals used in this study with their suppliers are listed in table (3.3).

Table (3.3): Kits and chemicals used in this study.

Materials	Supplier
Sodium chloride	Fluka, AG, Switzerland
Salivary alpha amylase assay ELISA kit	SALIMETRICS –USA, catalog no.1-1902

*ELISA: Enzyme-Linked Immuno-Sorbent Assay

3.4.2 Equipment and instruments

1. ELISA reader (Beijing Prolong New Technology. CO. LTD- Japan) as shown in Figure (3.1).
2. ELITE ion selective electrode reader (Elektra Medical Corporation-USA) as shown in Figure (3.5).
3. Electronic sensitive balance (AND GX-200-Japan)

4. Centrifuge (Remi motors, China).
5. Water bath (Haak, WB22-Germany).
6. Micropipette (5 - 200 µl) (Rainin, USA).
7. Multichannel micropipette (100 µl) (Japan).
8. Sterile graduated plane tubes for saliva and serum samples collection.
9. Disposable micropipette tips.
10. Graduated plane tubes for saliva and blood samples collection.
11. Eppendorff tubes (1.5 ml) for deep freeze sample storage.
12. Glass funnel for the collection of saliva.
13. Schematzu HPLC reader.

Figure 3.1: ELISA reader (Beijing Prolong New Technology. CO. LTD- Japan).

* The photo taken by the researcher during testing of samples in the college of veterinary medicine/University of Mosul.

3.5 Biochemical assays of saliva

3.5.1 Measurement of alpha amylase in saliva

3.5.1.1 Measurement of alpha amylase concentration in saliva

Determination of SAA concentrations was estimated using ELISA kinetic SAA assay kit from Salimetrics (USA) as in figure (3.2).

Principle: The Salimetrics© SAA assay kit is a simple kinetic enzyme assay that measures the concentration of AA in a saliva sample by observing its action on a color-producing substrate over time (Wallenfels *et al.*, 1978), the AA substrate consists of 2-chloro-*p*-nitrophenol (CNP) attached to a linear chain of three glucose molecules (maltotriose). AA cleaves the bond between the CNP and the maltotriose, releasing free CNP, which has a yellow color that can be measured in a spectrophotometer.

Figure 3.2: Salivary Alpha Amylase kit (SALIMETRICS)
*The photo was taken by the researcher during testing of samples.

The amount of yellow color formed is directly proportional to the amount of AA activity present in the sample (Figure 3.3).

The optical density (OD) of the CNP formed is first measured at 1 min after addition of the substrate, followed by a second measurement at the 3 min time point. The change in OD over the 2-minute measurement period is then used to determine the amount of AA activity present in the sample. The optical density data are easily converted by a formula into a result expressed in units/mL, where 1 unit equals 1 μmol of product formed per minute.

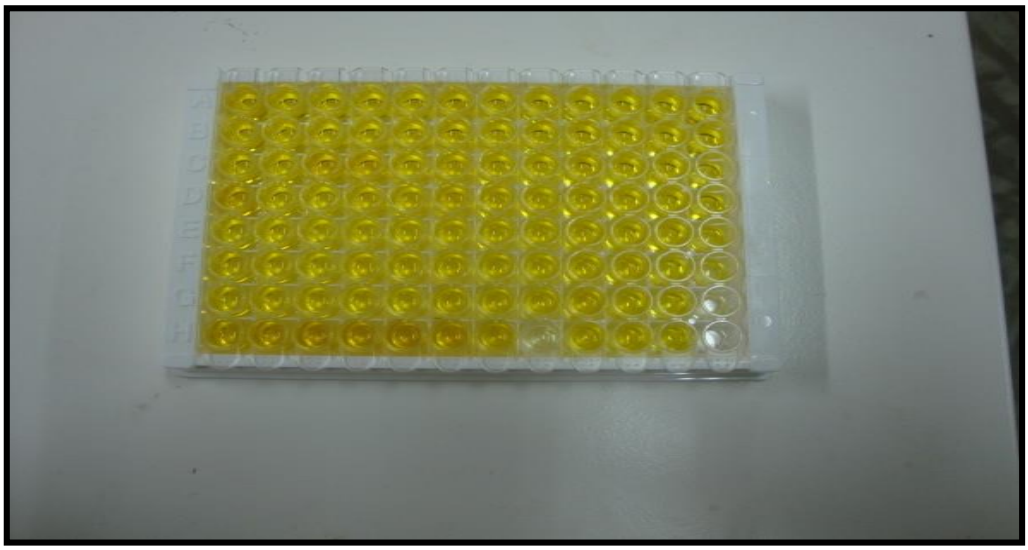

Figure 3.3: Yellow color formed in AA ELISA micro-titration.

*The intensity of the yellow color directly proportional to the amount of AA activity present in the sample. **The photo taken by the researcher during testing of samples.

Reagents

1. AA Substrate: 45 mL of a ready-to-use liquid preparation of 2-chloro-*p*- nitrophenol linked with maltotriose. Sodium azide, at 0.01%, is added as a preservative.
2. AA Controls: One vial containing 100 µL of a high level of SAA activity and one vial containing 100 µL of a low level of SAA activity in a saliva-like matrix. Controls come pre-diluted.
3. AA Diluent: 30 mL of a phosphate buffered solution containing a non-mercury preservative.

Preparation of Saliva Sample:

Saliva samples had to be diluted with the AA diluent provided. Prepare a 1:10 dilution of the saliva by pipetting 10 µL of saliva into 90 µL AA diluents. Mix well. Further dilute by pipeting 10 µL of the 1:10 dilution into 190 µL AA diluent (1:20). Final dilution is 1:200.

Procedures:

1. All reagent was thoroughly warmed and mixed before use. (A minimum warm-up time of 20 minutes, from room temperature, was recommended).
2. 8 µL of controls (prediluted) and/or diluted saliva samples were added to individual wells.

3. 320 µL of preheated (37ºC) AA substrate solution was added to each well simultaneously using a multichannel pipette.
4. Reading the result in 37ºC plate reader was conducted by doing the following steps:
    - The timer was started immediately and mix (500-600 RPM)
    - The OD at exactly 1 minute had been read and the plate was returned to mixing at 37ºC.
    - 1st minute OD readings were saved.
    - Then the OD at exactly 3 minute had been read.
    - 3rd minute OD readings were saved also.
5. Subtraction of the one minute readings from the three-minute reading were done, and the results of this subtraction were multiplied by the conversion. Excel spread sheet was used to subtract the ODs and multiply as shown in figure (3.4). Results were expressed in U/ml.

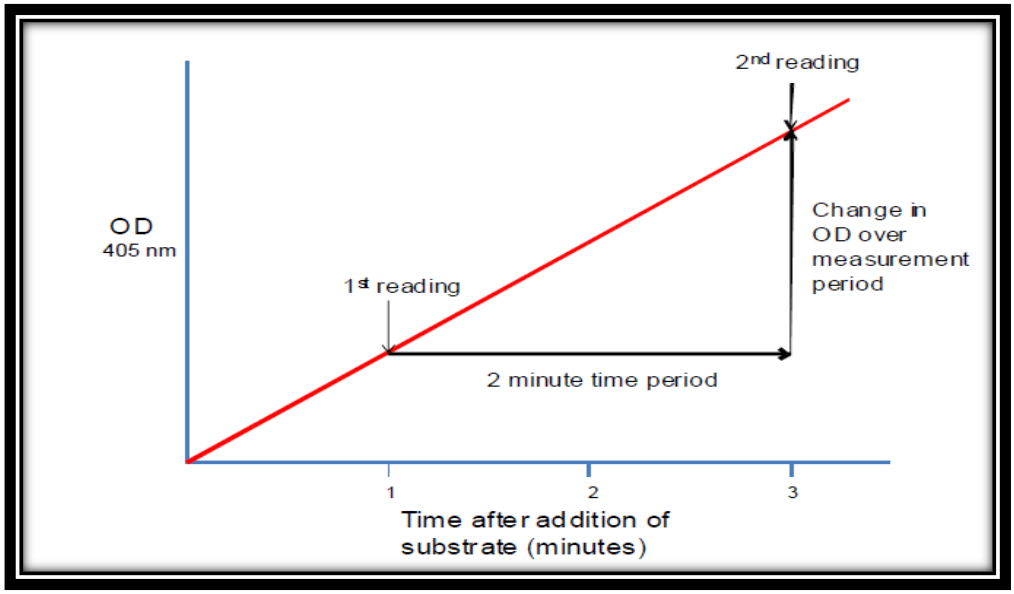

Figure 3.4: Excel spread sheet of SAA calculation

Calculations:

$$\frac{\Delta\textbf{Abs. /min} \times \textbf{TV} \times \textbf{DF}}{\textbf{MMA} \times \textbf{SV} \times \textbf{LP}} = \text{U/ml of SAA activity in saliva}$$

1. Where: ΔAbs./min = Absorbance difference per minute.
2. TV = Total assay volume (0.328 mL).

3. DF = Dilution factor (1:200).
4. MMA = Millimolar absorptivity of 2-chloro-p-nitrophenol (12.9).
5. SV = Sample volume (0.008 mL).
6. LP = Light path = 0.97(specific to plate received with kit).

$$\frac{\Delta \textbf{Abs. /2} \times \textbf{0.328} \times}{\textbf{200}} = \Delta \textbf{ Abs. x 328 = U/mL AA concentration}$$

3.5.1.2 Determination of SAA output in saliva

Many analytes was found to be affected by the salivary flow rate, such as SAA and secretary immunoglobulin A (SIgA) and others, thus its necessary to correct their concentrations with the salivary flow and obtained their salivary output of such analytes in u/min by multiplying it with the flow rate (Nederfor, 2000; Kaite et al., 2008) and as follows:

SAA Conc. (U/ml) × SF (ml/min) = SAA output (u/min)

3.5.2 Measurement of Na^+ and K^+ concentration in saliva

Na^+ and K^+ ions concentrations in saliva had been measured by the use of ELITE ion selective electrode (ISE) reader (ELEKTRA MEDICAL CORPORATION-USA) in the college of dentistry/University of Mosul as shown in (Figure 3.5).

Principle:

An ion selective electrode (ISE) generates a difference in electrical potential between itself and a reference electrode. The output potential is proportional to the amount or concentration of the selected ion in solution where the concentration means the measurement of the number of ions in a specific volume.

ELITE ISE is a microprocessor based instrument which was programmed with direct concentration modes of operation. Calibration and sample measurement are carried out automatically. Thus the traditional drawing of calibration graphs is no longer necessary.

Sample preparation:

According to ELITE reference manual, it is recommending to operate the general implications for serum, plasma, whole blood.

The range of performance of ELITE for the Na^+ and K^+ as analyte is out of the predicted concentration in our samples(saliva), thus our samples were prepared in a manner make its measurements by the ELITE ISE reader possible and reliable. A new method was created based on the basic principles of biochemistry that make the measurements of the salivary minerals (Na^+ and K^+ in saliva) is possible. This method was standardizing with flame photometry the traditional method of measuring Na^+ and K^+ concentrations for decade and accuracy was improved.

Figure 3.5: ELITE ion selective electrode (ISE) reader (ELEKTRA MEDICAL CORPORATION-USA)

3.5.2.1 Measurement of K^+ in saliva sample

As mentioned above, the range of performance of ELITE for the K^+ as analyte is (4-10mmole/liter), which is out of the predicted concentration in our samples (predicted to be 8-40 mmole/L according to many research like (Ritschel and Thombson,1983; Höld *et al*., 1999), the dilution method which is a known chemical method was used in the analytical procedures to make the measurement more reliable.

Sample Preparation: 0.5 ml of saliva sample diluted by 0.5ml of double deionized water and mix well. The sample then read by ELITE ion selective electrode reader and if the

concentration is too high, a second dilution (or third dilution, if needed) and good mixing after each dilution was ensured.

Calculations of K^+ Concentration: The concentration of K^+ was calculated as follows:

Concentration K^+ (mmole/ L) = reading mmole/ L × Dilution factor

3.5.2.2 Measurement of Na^+ in Saliva Sample

The range of performance of ELITE for the sodium ions as analyte was (60-200mmole/liter) which is also out of the predicted concentration in our samples (predicted to be 5-100 or even 150 mmole/liter) according to many researchs like (Ritschel and Thombson, 1983; Höld *et al*., 1999).

Sample Preparation: The addition subtraction method was done by adding a known concentration of a standard solution of Na^+ to the samples which is then subtracted from the ELITE reading was completed and as follows:

0.5ml of a standard Na^+ solution of (80 mmole/L) was added to 0.5 ml of saliva sample and well mixing was done and then measured.

Calculations: For sodium ion measurement, the concentration of sodium ion was calculated as follow:

Concentration of Na^+ (mmole/L) = (The reading of the ELITE in mmole/L - 40) × 2 (Dilution factor)

3.6 Determination of fluoxetine in serum by high performance liquid chromatography (HPLC)

3.6.1 Materials pure samples

Fluoxetine HCl was obtained from (Sigma Aldrich®), The purity given as % purity ± SD was found to be 100.65 ± 0.71.

3.6.2 Chemical and reagents

For HPLC work double distilled water was prepared in laboratory. Acetonitrile (Merck®), triethylamine (Sigma ChemicalCo., St. Louis, MO), phosphoric acid (Baker,

Phillipsburg, NJ), Methanol for chromatographic use (Merck®), chloroform, n-hexane and isopropanol were of HPLC grade from (Merck,Sharp & Dohme, West Point, PA). Ammonium chloride and 4-dimethylaminobenzaldehyde were

3.6.3 Specimen collection

Patients' blood samples were drawn in red-top Vacutainer blood-collection tubes (16 x 100 mm, 10-mL, no. 6530; Becton Dickinson, Rutherford, NJ). Specimens were allowed to clot and then centrifuged. Serum was removed from cells, aliquoted, and stored at -20 until analysis.

3.6.4 Instrumentation and chromatography

The HPLC system was a Shimadzu system comprising of a pump PU-2080 and a UV-2070 detector. Chromatography was conducted on a 5p.m (particle size), 15 cm x 4.6 mm LC-8-DB reversed-phase column. The mobile phase: 10mM triethylamine buffer pH 6.0 w/phosphoric acid/tetrahydrofuran/Methanol (for chromatographic use) (60: 30: 10) and acetonitrile, 65:35 (by volume), was filtered through a 0.45 p.m (pore size) nylon filter (Sigma Aldrich®) and degassed before use, operating at ambient temperature. Shimadzu AY 120 (max 120 g d = 0.1 mg) analytical balance was used for weighing, PCi Ultrasonicator, Laboratory centrifuge Remi R- 8C was used for centrifugation.

The therapeutical plasma concentrations of fluoxetine are very low (at a level < 1µg/mL). Therefore, a sensitive and selective method for the assay of fluoxetine is needed.

The chromatographic conditions were as follow: the flow rate was set at 1.5 ml/min, detection wavelength at 227 nm, injection volume was 10 µL and temperature was 22 C, analysis time: 3 minutes. Calibrators and stock standards were prepared and stored at -20ºC until use and were stable for at least 6 months under these conditions.

Standard solution was prepared: Fluoxetine HCl-110µg/mL in mobile phase (fluoxetine stock solution: 10 mg of fluoxetine hydrochloride were quantitatively transferred into a 10 mL volumetric flask, dissolved in (solvent) and diluted to volume with the same solvent. Fluoxetine working solution (10µg/mL) was prepared by dilution with mobile phase, from the stock solution).

Peaks were detected at 227 nm on a Shiniadzu SPD-6AV ultraviolet spectrophotometric detector set to a sensitivity of 0.02 absorbance unit (full scale) with normal response time. The amount of drug was quantified by the peak height ratio of analyte to standard.

3.7 Statistical analysis

Statistical analysis of data was conducted using SPSS 17 for windows software. A p-value of < 0.05 was considered statistically significant. The following statistical methods were used for the analysis of data (Runyon, 1977; Howitt and Cramer, 1999):

- The results for age, sex, dose, duration, and biochemical parameters in saliva were expressed as mean ± standard deviation (SD).
- The results of clinical indications, general and oral adverse effects were expressed as the number of observations.
- The *t* test was used to analyze normally distributed data for the demographic and biochemical characteristics of the study population.
- Non-Parametric test assumptions were conducted for comparisons of these groups by means of the Mann-Whitney U test and Kruskal-Wallis test. The results of nonparametric tests were expressed as the number of observations and means ± standard deviation. Spearman rank correlation coefficient was used to evaluate correlations between non parametric variables and Pearson correlations between parametric variables.

In this study, first of all, a comparison was made between the pretreated level (base line level) of follow up group with the control group, any deviation from normal condition clearly indicated the effect of the disease (depression, OCD, anxiety, panic disorders, and postpartum depression) on the patients.

A second comparison was done between the chronic group and the pretreated (base line level) of follow up group and the control group, and this will have explained the effect of fluoxetine therapy on the patients (long term therapy).

A third comparison was done in the follow up group parameters, between pretreated base line level and the post 1 and post 2 parameters, after 4weeks and 8weeks of treatment with fluoxetine in the acute group. This indicated the pure effects of the fluoxetine therapy on the patients.

4. RESULTS

4. RESULTS

4.1 Patients characteristics

4.1.1 The demographic distribution of patients age and gender between the groups

The number of individuals enrolled in this study was 80, their average age was (35.25 ± 12.16) years, consisted of (40 M) and (40 F), their average age was (35.88± 12.75) and (34.63± 11.57) years respectively as shown in Table (4.1). The eighty individuals were distributed in two major groups:

1. Patients group (Acute and Chronic groups)
2. Control group

Table 4.1. Demographic distribution of (age and gender) between groups in the study.

Groups	Gender	N	Age(years) mean± SD
Acute	M	23	35.2±11.7
	F	16	35.8±9.6
	Total	39	35.4±10.7
Chronic	M	7	36.4±12.5
	F	14	32.7±10.0
	Total	21	33.95±10.7
Control	M	10	36.0±14.0
	F	10	35.3±14.5
	Total	20	35.6±13.9
Total no.	M	40	35.8±12.7
	F	40	34.6±11.5

1. Patients group:

A total of (60) patients (30 M and 30 F) suffering from different psychiatric conditions were selected to participate in the study. Their age between (15-56) years. The age distribution was shown in figure (4.1)

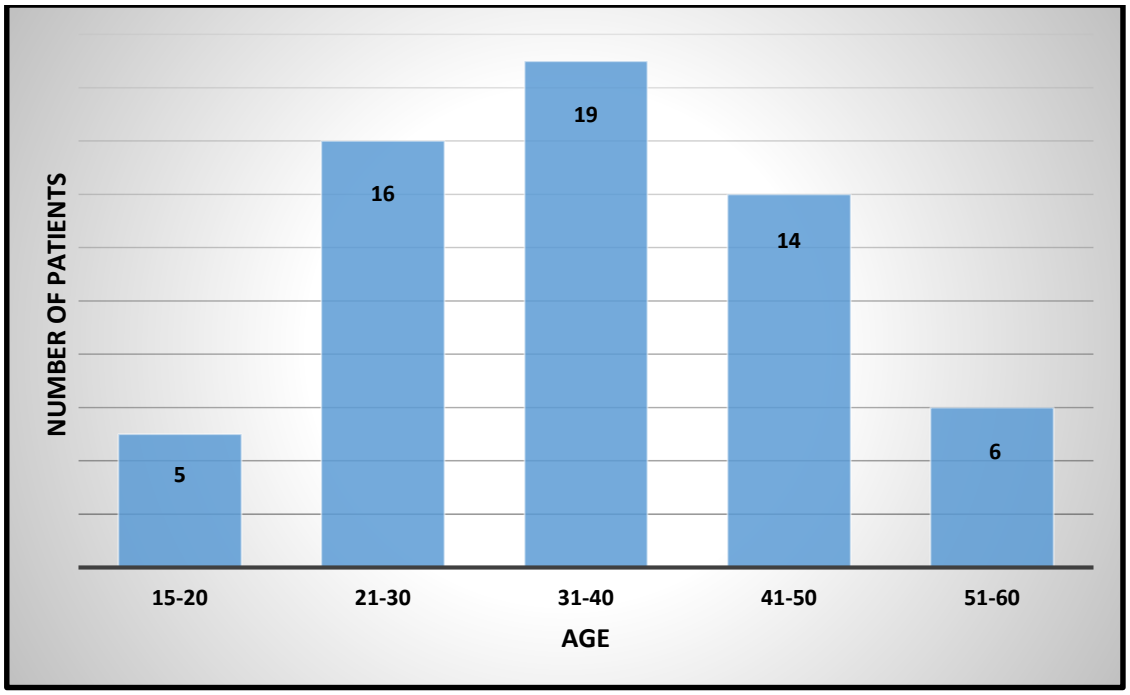

Figure 4.1: Frequency distribution of age in the study group.

A. Acute group:

Thirty nine patients were selected to receive fluoxetine capsules in a dose ranging from (20 to 60 mg/day). Their sex distribution and age were shown in table (4.1). The dose adjustment (increased or decreased) in each visit was based on the safety and toleralability of the drug on the patients as shown in table (4.2).

Table 4.2 : The number and percentage of patients in the acute and chronic group with the doses of fluoxetine administered:

Dose (mg/day)	Acute			Chronic
	Pre	Post 1	Post2	
20	39(100%)	32(100%)	13(54.2%)	8(38%)
40	0(0%)	0(0%)	7(29.2%)	13(61.9%)
60	0(0%)	0(0%)	4 (16.6%)	0(0%)
Total No	39(100%)	32(100%)	24(100%)	21(100%)

B. Chronic group:

Twenty one patients were investigated including (7 M) , (average age 36.43±12.5 years) and (14 F) (average age 32.71±10.0 years) as mentioned in table (4.1). Single reading was taken in this group. Table (4.2) showed different dosage regimes used by patients of this group.

2. Control group :

Twenty healthy subjects were asked to enrolled in this study (average age 35.65±13.9 years) consisted of (10 M) and (10 F) with their average age (36.0±14.0) and (35.30±14.5) years respectively as shown in table (4.1). The same parameters were collected from each subject.

4.1.2 Clinical indications of fluoxetine

Fluoxetine had been prescribed for different psychatric disorders in this study including:

1. Depression (mild, moderate, severe, and chronic depression).
2. Anxiety disorders .
3. Obssesive compulsive disorders (OCD) .
4. Panic disprders and
5. Post partum depression.

The total number of patients and number of M and F (and their percentages) which had been daignosed in each clinical indications were shown in Table (4.3).

Table 4.3: Clinical indications of fluoxetine in the study.

Gender	Depression No. (%)	Anxiety No. (%)	OCD No. (%)	Panic disorder No. (%)	Postpartum depression No. (%)	Total No. (%)
Female	26 (43.3%)	2 (3.3%)	0 (0%)	1 (1.7%)	1 (1.7%)	30(50%)
Male	19 (31.6%)	8 (13.3%)	2 (3.3%)	1 (1.7%)	0 (0%)	30(50%)
Total	45 (74.9%)	10 (16.6%)	2 (3.3%)	2 (3.4%)	1 (1.7%)	60(100%)

Many life factors may contribute in the psychological disorders or the continuation of the disease and may make the subject suffer from stress leading to depression and other psychological disorders such as age, gender, marital state, person occupation or no, studying or no, getting the good marks in exams, getting the appropriate work, family and children and many other think that important for human life. As can be seen in Figure 4.2.

Fifty percent of the sample population was male and the rest was female.

Eight percent of the patients is teenager while the large percent was adults 59% (27% under age 30 and 32% under 40) and only 10% in the age of retirement.

Forty-one percent of the patients was under the stress of working and have different types of work and thirty seven percent was under the stress of disemployment and trying to find a work to live.

Seventen percent of the patients was students under stress of studying and examination. A small percent of only (5%) was retired persons.

Forty-seven percent of the patients was married, 30% single and 27% was divorce.
In the control group, we try to get person on the same conditions of the patients (age, sex, marital state, occupation) as possible as to make the comparison rasonable. As try to get persons in the same life style of the patients. And this very clear in Figure (4.2).

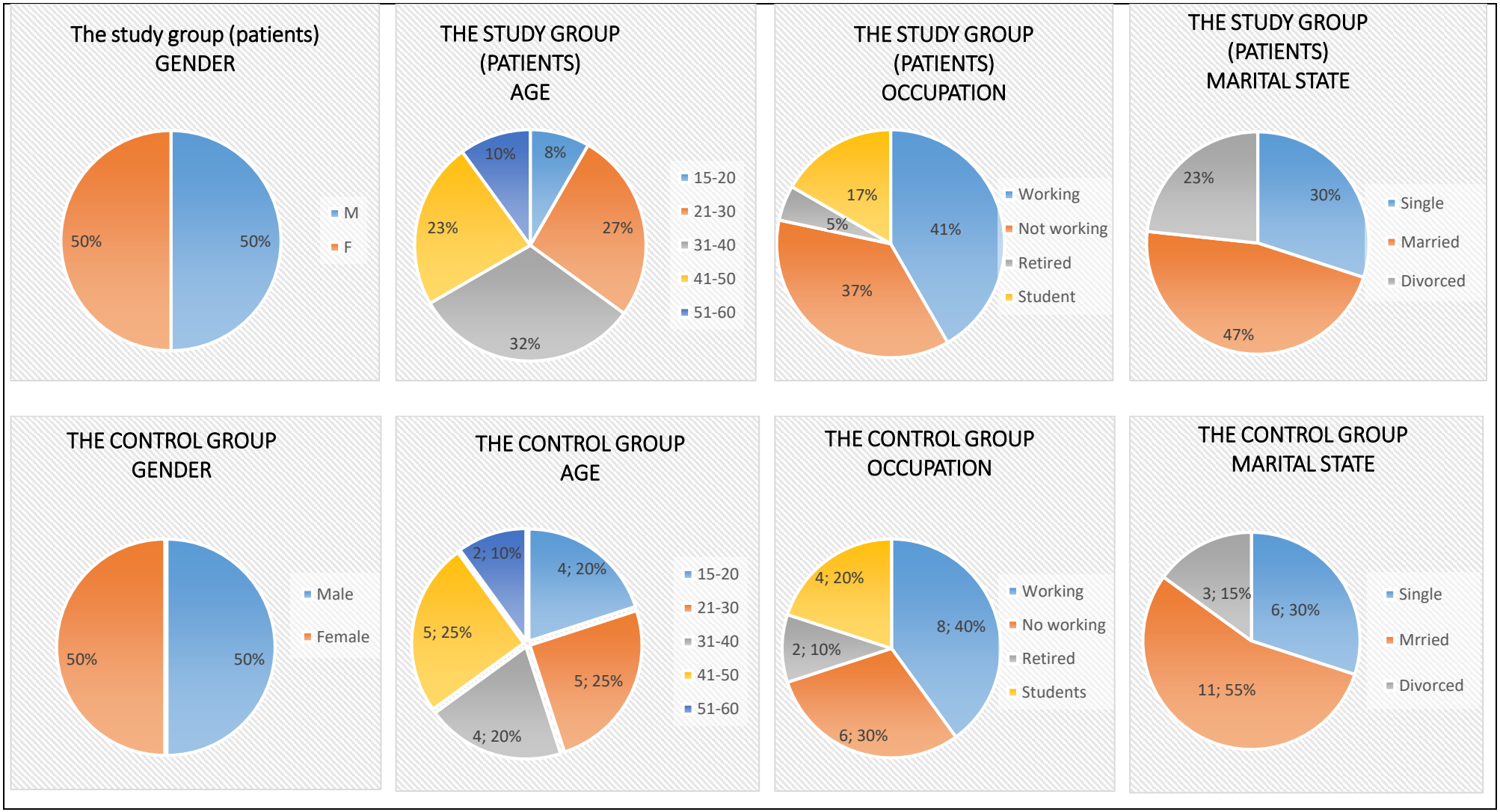

Figure 4.2: Percentage of gender, age, occupation, marital state in the study group (patients) and control group

4.2 Salivary alpha amylase concentrations as a possible biological marker of stress in psychological patients

The SAA conc.(U/ml) was measured in the three groups as seen in Table (4.4)

Table 4.4: The salivary alpha amylase obtained from all groups

Groups		SAA conc. (U/ml)	SAA output (U/min)
Acute	pre	40.31±26.6	19.87±14.9
	Post1	41.57±29	12.86±16.7
	Post2	54.07±29.9	11.43±17.8
Chronic		70.99±52.8	20.46±23.2
Control		12.27±3.5	10.97±6.9

4.2.1 Comparison of SAA activity (concentration and out put) between groups

Significant difference reported between the three groups at $p < 0.05$. The chronic group concentration (70.99±52.8)U/ml was significantly higher than the pre level (40.31± 26.6)U/ml and the control group (12.27±3.5) U/ml as shown in table (4.5).

Table 4.5: Comparison of SAA between Groups

Salivary parameters	Acute (Pre) n=39	chronic n=21	control n=20	P= value
SAA Conc. (U/ml)	40.31±26.6 a	70.99±52.8 b	12.27±3.5 c	0.0001
SAA Output (U/min)	19.87±14.9 a	20.46±23.2 b	10.97±6.9 c	0.000

*Data represened as mean ±SD.

**Different letters (a, b, c) horizontaly means significant differences at $p<0.05$.

The significant differences founded between SAA level in the chronic and healthy state may indicate that it can be used as a biomarker of stress in the chronic conditions. The comparison between the three groups in their SAA concentrations showed increased level

of SAA in disease condition (representing here by the chronic group and the pretreated level) than the control healthy subject in the control group.

The concentration of SAA in the chronic group show increments (478.5%) than the control level and only (76.1%) than the pre treated level. While, the pre level increment than the control level was (228.5%).

SAA output showed Significant difference between all groups at $p<0.05$ as shown in Table (4.5). The chronic group showed significantly higher SAA output (20.46± 23.2)U/min than the control group (10.97±6.9)U/min while it was slightly higher than the pre treated level (19.87±14.9) U/min.

SAA output as SAA conc. was higher in disease condition (representing by the chronic and pre level of acute group) than in the healthy condition

The percentage of increment of SAA out put in the chronic group was (86.5%) than the control group and it was only (2.9%) than the pre level. While the pre level increment than the control level was (81.1%).

4.2.2 Comparison of SAA activity (concentration and out put) between pre, post 1 and post 2 readings in the acute group

Although there were no significant differences $P>0.05$ between the three readings of SAA conc. in this group as shown in Table (4.6). But, the concentration of SAA in post2 (54.07±29.9)U/ml was higher than post1 (41.57±29.0)U/ml and the lowest concentration reported in pretreated base line level (40.31±26.6)U/ml in this group as can be seen in Table (4.4).

The percentage of increment of post 2 SAA conc. over the pre treated level was (34.1%) and (30%) over the post 1 which increased over the pre level by only (3.1%).

Table 4.6 : N-Pair test of SAA concentration and output for the acute group:

Salivary parameter	Pre-post1 P value	Pre-post2 P value	Post 1- post 2 P value
SAA conc. (U/ml)	NS	NS	NS
SAA output (U/min)	NS	0.048	0.040

*NS means no significant differences at $p<0.05$.

Significant difference had been shown between the reading of patients (after fluoxetine therapy) and pre treated level at p value<0.05 in SAA output as shown in table (4.6). The SAA output baseline pre treated level (19.87±14.9)U/min was significantly higher than post2 level (11.43±17.8)U/min. Also post2 level was significantly lower than post1 level. The SAA output appeared to decrease with fluoxetine therapy duration as shown in the decline in SAA output in post2 (after 8weeks of fluoxetine therapy)than in post1(4 weeks of therapy). The percentage of post 2 level decline was (42.4%) than pre treated level and only (11.1%) than the post 1level which decline from the pre level by only (25.2%) .

From all these comparison, we can notice that SAA was higher in the stress condition (in patient's group; acute and chronic) than in the control healthy subjects which make it a good indicator of stress as it clear notice in Figure (4.3).

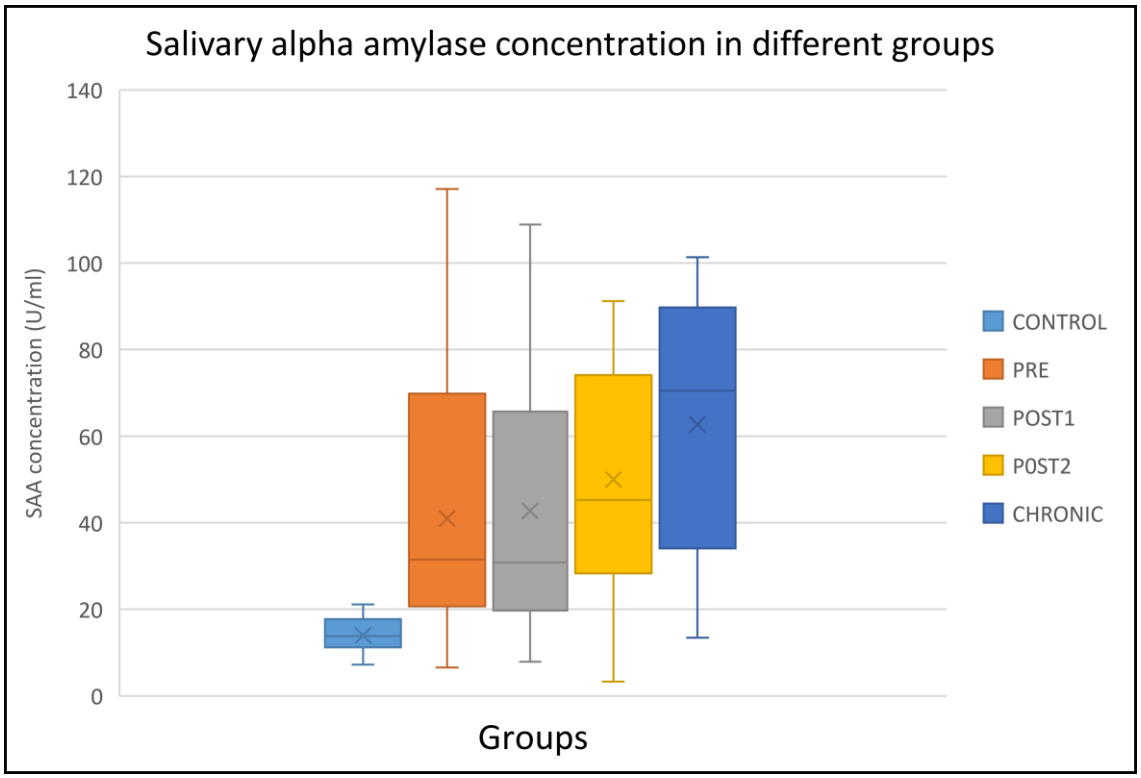

Figure 4.3: Salivary alpha amylase concentrations in groups.

Since we have indicated that SAA could be used as a biological marker of stress, let us study its efficiency to detect psychological patients under stress, and since there is no one universal normal salivary alpha amylase level published and accepted all over the world and thus we try made a comparison considering the measurements in the healthy subjects (the control group) as the distinct or cutoff value between healthy and disease

condition, especially that all living under the same conditions. Considering the levels in the healthy control subjects as a normal value at rest in our sample of population. For example, concerning the pretreated patients, it was noticed that 30 of 39 patients have SAA level higher than the highest SAA level in the healthy control group. This mean that 76.9% of the psychological patients under stress can be detected by this simple SAA test. Table (4.7) shows the detection percentage of stress in psychological patients in groups by using SAA concentration. It can be seen that percentage of detection of stress is high and thus we beleive that it's a good percent of detection which ensure that SAA is a good biomarker of stress in psychological patients.

Table (4.7): Percentage of detection of stress in psychological patients in groups by using SAA concentration

Groups		No. of patients	Percentage of patients under stress(higher SAA level than control healthy condition)
Acute	pre	30	76.9%
	Post1	26	81.2%
	Post2	22	91.6%
Chronic		18	85.7%

4.3 Na^+ and K^+ concentrations in saliva in acute and chronic groups

4.3.1 Comparison of sodium and potassium ion concentrations between groups

Significant difference was shown in the concentration of Na^+ between the pre treated level and control group while the chronic group showed no significant differences with both the pre level and control group. The concentration of Na^+ in the chronic group (51.33±8.7)mmol/l was higher than the control group (48.30±7.6)mmol/l but it was less than pre treated concentration (54.76±7.3)mmol/l .

Generally Na^+ concentration was higher in the diseased state than in the healthy state. Also significant differnce was shown in K^+ concentration between the three groups at $p< 0.05$ as shown in Table (4.8). The pretreated level (28.31± 20.4) mmol/l was significantly lower than both the chronic and control levels (40.85±21.0 and 46.43± 24.8) mmol/l

respectively. Although no significant differences between control and chronic groups, but the concentration of K^+ was higher in the control group than in the chronic group as can be seen in table (4.8).

Table (4.8): Comparison of sodium and potassium ion concentrations between groups.

Salivary parameters	Acute(Pre) n=39	Chronic n=21	Control n=20	P value
Na^+ (mmol/l)	54.76±7.3 a	51.33±8.7 a b	48.30±7.6 ь	0.01
K^+ (mmol/l)	28.31± 20.4 a	40.85±21.0 ь	46.43± 24.8 ь	0.007

*Data represened as mean ±SD.

**Different letters (a,b) horizontaly means significant differences at p<0.05.

4.3.2 Comparison of Na^+ concentration(mmol/l) between pre, post 1 and post 2 readings in acute group

The pretreated level of Na^+ (54.77±7.3) mmol/l was higher than post1 and post2 concentration (49.92± 5.4 and 47.88±7.5) mmol/l respectively as shown in table (4.9).

Table 4.9 : Changes of sodium concentration in the acute group.

Salivary parameters	Pre n=39	Post1 n=37	Post2 n=24
Na^+(mmol/l)	54.77±7.3	49.92± 5.4	47.88±7.5
K^+ (mmol/l)	28.32±20.4	33.83±26.1	38.27±36.5

*Data represened as mean ±SD. **Significant at p value ≤0.05.

Although no significant difference was reported between these two readings (post1 and post2) but, the concentration in post2 (two month duration of therapy) was lower than post1. There was a (12.5%) decline in the concentration of Na^+ in post 2 than the pretreated level and (4.0%) than post 1 level while post 1 decline than the pretreated level by only (8.8%).

K^+ concentration showed no significant difference in in this group at $p< 0.05$. But the concentration was higher in the post 2 readings (38.27±36.5) than both post1 and the pre treated level (33.83±26.1) and (28.32±20.4) respectively. We can notice that the lowest level was reported in the pre treated reading. K^+ concentration showed increment with increased duration of fluoxetine therapy

Making the same procedure of comparison made on SAA activity concerning the Na^+ and K^+ concentration measurements and comparing it with the healthy levels, we have notice that there is a difference in the concentration between healthy and disease state but this differences is not sufficient to make a clear distinction between healthy and disease state. The percentage of detection of stress in our sample population using salivary sodium and potassium conc. was low. Thus we think that salivary Na^+ and K^+ measurements cannot be used solely as biomarkers and it might need more studies. This can be deduced from Figures (4.4), and (4.5).

Sodium show increment in the acute disease condition (sodium conc. in the pretreated level was higher than the control level) and decreased gradually with treatment while show different pattern of response in the chronic state. While potassium conc. show decrease in the disease state, the pretreated level was lower than the healthy state and this concentration increased with treatment as can be seen in Figure (4.5). Post1 and post 2 levels was higher than the pretreated level before administration of fluoxetine therapy.
Using the Na^+ concentration measurement, only 4 pretreatment patients are detected by the comparison of their Na^+ concentration with the healthy subjects, whilst using the K^+ concentration measurement only 3 pretreatment patients (diagnosed psychological patients before using fluoxetine therapy) are detected.

And by using the SAA, Na^+ and K^+ concentration measurements together to detect psychological patients, the number of pretreated patients that has been detected increases from 30 to 32 from the original 39 patients, 82.05% of the total patients can be detected which consider a good high percent of detection and might consider them as a good possible biomarkers of stress.

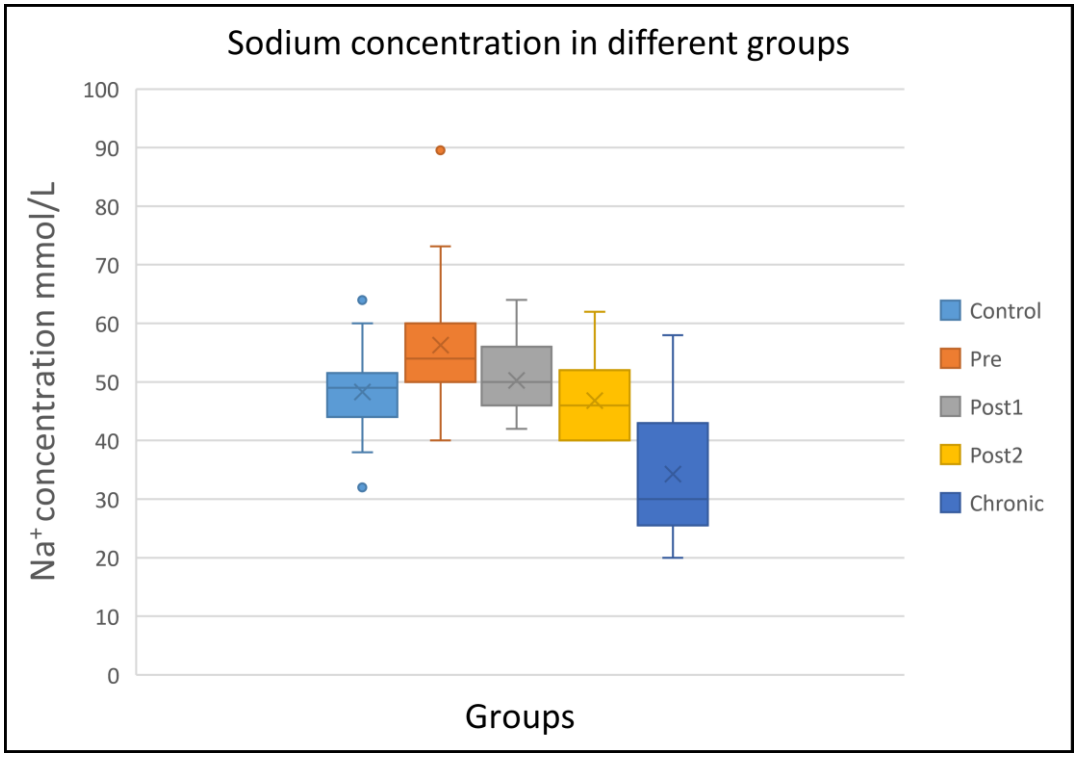

Figure 4.4: Salivary sodium concentrations in groups.

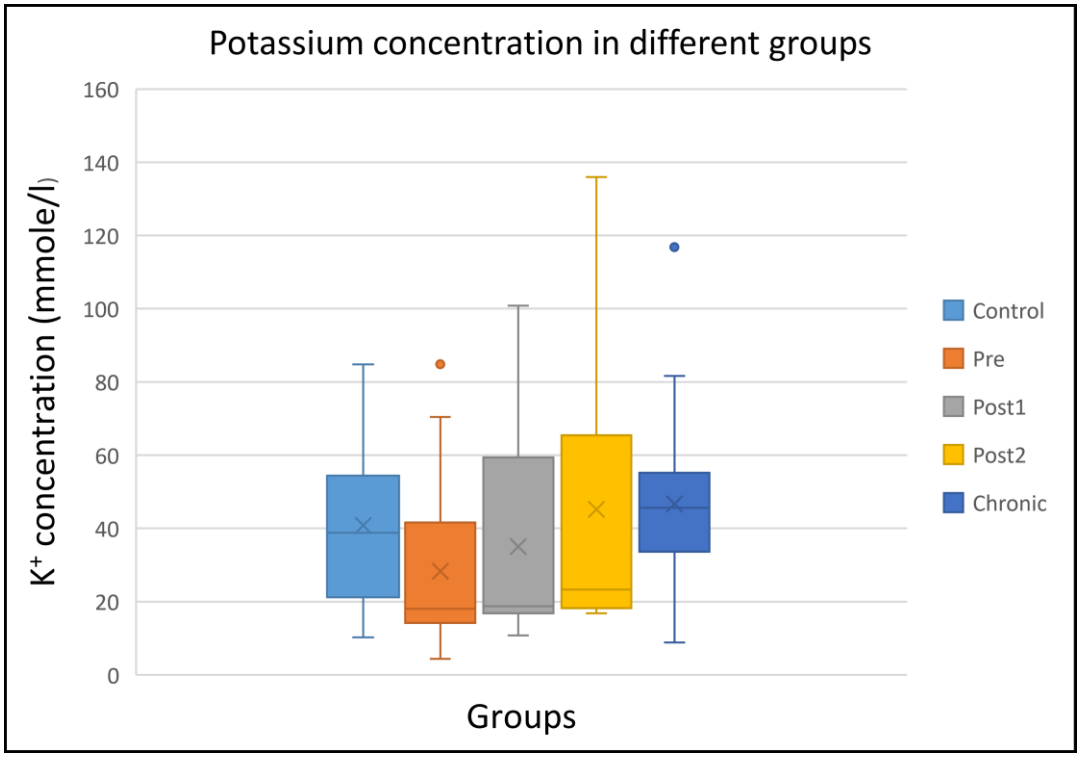

Figure 4.5: Salivary potassium concentrations in groups.

4.4 Assessments of the effects of fluoxetine therapy on the salivary parameters obtained from the participants

The comparison was done for all the biochemical and clinical parametres collected from patients Table (4.10).

Table 4.10: The salivary parametres obtained from all groups

Groups		SAA conc. (U/ml)	SAA output (U/min)	Na^+ conc. (mmol/l)	K^+ conc. (mmol/l)	FR (ml/min)	VAS
Acute	pre	40.31±26.6	19.87±14.9	54.77±7.3	28.32±20.4	0.46±0.2	17.51±12.5
	Post 1	41.57±29	12.86±16.7	49.92±5.4	33.83±26.1	0.36±0.17	24.91±10.0
	Post 2	5 4.07±29.9	11.43±17.8	47.88±7.5	38.27±36.5	0.32±0.14	29.05±10.3
Chronic		70.99±52.8	20.46±23.2	51.33±8.7	40.85±21	0.32±0.22	29.05±9.2
Control		12.27±3.5	10.97±6.9	48.3±7.6	46.43±24.8	0.56±0.17	8.65±2.7

*Data represened as mean±SD

First, the comparism was done between the three groups (control, chronic and the pretreated level of the acute group). This comparism show the differences in the disease condition (treated and untreated) and the healthy condition and explain the effects of psychiatric disease on general health of the patient.

4.4.1 Comparison of salivary parameters between the three groups (chronic and pretreated level and control group)

4.4.1.1 Comparison of salivary biochemical parameters (SAA, Na^+, K^+ concentrations) between the three groups

Comparison of SAA concentration (U/ml) between groups: Significant difference reported between the three groups at $p<0.05$. The chronic group concentration (70.99±52.8) was significantly higher than the pre level (40.31± 26.6) and the control group (12.27±3.5) as shown in table (4.5), menthioned above.

Comparison of SAA output (U/min) between groups: SAA output showed Significant difference between all groups at $p<0.05$ as shown in Table (4.5). The chronic group showed significantly higher SAA output (20.46± 23.2) than the control group (10.97±6.9) while it was slightly higher than the pre treated level (19.87±14.9). SAA output as SAA conc. was higher in disease condition (representing by the chronic and pre level of acute group) than in the healthy condition.

The percentage of increment of SAA output with fluoxetine administration in the chronic group was (86.5%) than the control group and it was only (2.9%) than the pre level. While the pre level increment than the control level was (81.1%).

Comparison of Na+ concentration (mmol/l) between groups: Significant difference was shown in this study between groups in concentration of Na+ in saliva at p value <0.05 as shown in Table (4.11).

Table 4.11: Comparison of Na+ concentrations between groups.

Salivary parameters	Acute(Pre) n=39	Chronic n=21	Control n=20	P value
Na^+ (mmol/l)	54.76±7.3 a	51.33±8.7 a b	48.30±7.6 ь	0.01

*Data represened as mean ±SD.

**Different letters (a,b) horizontaly means significant differences at $p<0.05$.

Significant difference was shown in the concentration of Na^+ between the pre treated level and control group while the chronic group showed no significant differences with both the pre level and control group. The concentration of Na^+ in the chronic group (51.33±8.7) mmol/l was higher than the control group (48.30±7.6) mmol/l but it was less than pre treated concentration (54.76±7.3) mmol/l . It is clear that the concentration of Na^+ was decline with the treatment as shown from the concentration of the chronic group which was less than the pre treated level in disease condition, but it was still higher than the control group.

The pretreated level was increased than the control level in (12.4%) while, the chronic level decline with fluoxetine treatment by (6.6%) than the pretreated level, but still higher than the control level by (6.2%). Generally Na^+ concentration was higher in the diseased state than in the healthy state.

Comparison of K^+ concentration (mmol/l) between groups: Significant differnce was shown in K^+ concentration between the three groups at $p< 0.05$ as shown in Table (4.12). The pretreated level (28.31± 20.4) mmol/l was significantly lower than both the chronic and control levels (40.85±21.0 and 46.43± 24.8) mmol/l respectively. Although no significant differences between them, but the concentration of K^+ was higher in the control group than in the chronic group.

Table 4.12: Comparison of potassium ion concentrations between groups.

Salivary parameters	Acute(Pre) n=39	Chronic n=21	Control n=20	P value
K^+ (mmol/l)	28.31± 20.4 a	40.85±21.0 ь	46.43± 24.8 ь	0.007

*Data represened as mean ±SD.

**Different letters(a,b) horizontaly means significant differences at $p<0.05$.

Concentration of K^+ showed increment with fluoxetine treatment as can be shown from the concentration of chronic group which was higher than the pre level in the disease condition by (44.1%) but this concentration was still less than the control level by (12%). The disease level represented by pre level was (39%) less than control level. Generally K^+ concentration was less in the diseased state than in the healthy state.

4.4.1.2 Comparison of the salivary flow rate and visual analoug scale between the three groups

Significant difference was shown between the three groups in the salivary flow rate (FR) and visual analogue scale (VAS) at $p< 0.05$ as shown in table (4.13).

The FR was higher in the healthy subjects than in patients, while VAS showed higher scores in patients rather than control healthy subjects.

A good correlation can be noticed betwen salivary flow and VAS which gave a good patient express of mouth dryness, make VAS a good scale for mouth dryness.

Table 4.13: Comparison of Flow Rate and Visual Analog Scale between the three groups:

Salivary parameters	Acute (Pre) n=39	Chronic n=21	Control n=20	P value
FR (ml/min)	0.46± 0.20 a	0.32±0.22 ь	0.56± 0.17 a	0.002
VAS	17.51± 12.5 a	29.05±9.2 ь	8.65± 2.7 c	0.000

*Data represened as mean ±SD.

* *Different letters (a,b,c) horizontaly means significant differences at p<0.05.

Comparison of salivary flow rate between the three groups: As mentioned in table (4.13), the chronic group showed significant difference from both the control and pretreated level while no significant difference reported between these two levels at p<0.05. The disease condition represented by (the chronic level and the pre treated level) had showed lower flow rate (0.32±0.22) and (0.46±0.20) ml/min respectively than the healthy condition (control level)(0.56± 0.17)ml/min which was the higher reported level.

Comparison of Visual Analog Scale between the three groups: As can be seen in table (4.13), significant difference was reported between the three groups at p<0.05. The chronic group showed significantly higher score (29.05±9.2) than both the pre treated level (17.51± 12.5) and the control group (8.65± 2.7) which reported the lower score between the three groups. This may indicate increase patient sensation of mouth dryness in the disease condition and also the sensation of dryness increased with time with the fluoxetine therapy as clear in the chronic group score.

4.4.2 Changes in the salivary parameters in the acute group (pre, post1, post 2 readings)

The comparism have been done between the three readings of the acute group, before starting fluoxetine therapy, post 4 weeks and 8 weeks of fluoxetine administration which might demonstrate the effects of fluoxetine therapy on the patients.

4.4.2.1 Changes in the salivary biochemical parameters (SAA, Na^+, K^+ concentrations) in the acute group

Comparison of SAA concentration(U/ml) between pre, post 1 and post 2 readings: There were no significant differencesp>0.05 between the three readings of SAA conc. in this group as shown in table (4.14).

Table 4.14: N-Pair test of SAA concentration and output for the acute group :

Salivary parameter	Pre-post1 P value	Pre-post2 P value	Post 1- post 2 P value
SAA conc. (U/ml)	NS	NS	NS
SAA output (U/min)	NS	0.048	0.040

NS means no significant differeces between data.

**Significant at p value ≤0.05.

The concentration of SAA in post 2 (54.07±29.9) was higher than post1 (41.57±29.0) and the lowest concentration reported in pretreated base line level (40.31±26.6) in this group as can be seen in Table (4.15).

The percentage of increment of post 2 SAA conc. over the pre treated level was (34.1%) and (30%) over the post 1 conc. which increased over the pre level by only (3.1%).

Table 4.15 : Changes of SAA in the acute group.

Salivary parameters	Pre n=39	Post1 n=37	Post2 n=24
SAA Conc. (U/ml)	40.31±26.6	41.57±29.0	54.07±29.9
SAA Output (U/min)	19.87± 14.9	12.86±16.7	11.43±17.8

* Data represened as mean ±SD. **Significant at p value ≤0.05.

Comparison of SAA output (U/min) between pre, post 1 and post 2 readings: Significant difference had been shown between the reading of patients (after fluoxetine

therapy) and pre treated level at p value<0.05 as shown in table (4.14). The baseline pre treated level (19.87± 14.9) was significantly higher than post2 level (11.43±17.8). Also post2 level was significantly lower than post1 level.

The SAA output appeared to decrease with fluoxetine therapy duration as shown in the decline in SAA output in post2 (after 8 weeks of fluoxetine therapy) than in post1(4 weeks of therapy). The percentage of post 2 level decline was (42.4%) than pre treated level and only (11.1%) than the post 1level which decline from the pre level by only (25.2%) .

Comparison of Na^+ concentration(mmol/l) between pre, post 1 and post 2 readings: The pretreated level (54.77±7.3) mmol/l was higher than post1 and post2 concentration (49.92± 5.4 and 47.88±7.5) mmol/l respectively as shown in table (4.16).

Table 4.16 : Changes of sodium concentration in the Acute Group:

Salivary parameters	Pre n=39	Post1 n=37	Post2 n=24
Na^+(mmol/l)	54.77±7.3	49.92± 5.4	47.88±7.5

*Data represened as mean ±SD. **Significant at p value ≤0.05.

Although no significant difference was reported between these two readings (post1 and post2) but, the concentration in post2 (two month duration of therapy) was lower than post1. There was a (12.5%) decline in the concentration of Na^+ in post 2 than the pretreated level and (4.0%) than post 1 level while post 1 decline than the pretreated level by only (8.8%). Significant difference was reported in the concentration of Na^+ ion between the pre treated level and post1 and post 2 at $p < 0.05$ as shown in table (4.17).

Table 4.17: N-Pair test of Na^+, K^+ concentrations for the Acute Group

Salivary parameters	Pre-post1 P value	Pre-post2 P value	Post 1- post 2 P value
Na^+(mmol/l)	0.000	0.000	NS
K^+(mmol/l)	NS	NS	NS

*NS means no significant differeces between data.

**Significant at p value ≤0.05.

Comparison of potassium concentration (mmol/l) between pre, post 1and post 2 readings: No significant difference in K^+ concentration in this group at $p< 0.05$ as shown in table (4.15). But the concentration was higher in the post 2 readings (38.27±36.5) than both post1 and the pre treated level (33.83±26.1) and (28.32±20.4) respectively. As shown in table (4.18).

Table 4.18: Changes of K^+ concentrations in the Acute Group.

Salivary parameters	Pre n=39	Post1 n=37	Post2 n=24
K^+ (mmol/l)	28.32±20.4	33.83±26.1	38.27±36.5

*Data represened as mean ±SD. **Significant at p value ≤0.05.

We can notice that the lowest level was reported in the pre treated reading. K^+ concentration showed increment with increased duration of fluoxetine therapy, (35.1%) increment in post 2 level than the pretreated level and (13.3%) than post 1 K^+ concentration. While post 1 level increased by (19.4%) than the pre treated level.

4.4.2.2 Changes of the salivary flow rate and visual analoug scale between pretreated, post 1 and post 2 readings of the acute group

Comparison of salivary flow rate between pretreated, post 1 and post 2 readings of the acute group: The FR decreased with fluoxetine therapy and this decrease can be shown in the higher FR reported before starting fluoxetine administration and lower FR reported after two months of therapy as can be seen in table (4.19).

Table 4.19 : Changes of salivary FR and VAS in the acute group.

Salivary parameters	Pre n=39	Post1 n=37	Post2 n=24
FR (ml/min)	0.46±0.20	0.36±0.17	0.32± 0.14
VAS	17.51±12.5	24.91±10.0	29.05±10.3

*Data represened as mean ±SD.

The FR was significantly high in the pretreated baseline level (0.46±0.20) than both post1 and post2 levels which were (0.36±0.17) and (0.32± 0.14) respectively. No significant difference reported between post1 and post2 which reported the lowest salivary FR in this group as can be seen in table (4.20).

Table 4.20: N-Pair test of SFR and VAS for the acute group :

Salivary parameters	Pre-post1 P value	Pre-post2 P value	Post 1- post 2 P value
FR(ml/min)	0.01	0.006	NS
VAS	0.000	0.000	NS

Comparison of visual analouge scale between Pretreated, Post 1 and Post 2 readings of the acute group: In this group, the VAS showed significant difference at $p < 0.05$ between untreated (pre level) and treated levels (post1 and post2) as shown in table (4.20). The VAS score which was significantly lower in the pre treated base line level (17.51±12.5) than both post treatment readings (24.91±10.0) in post1 and (29.05±10.3) in post 2 .

Although no significant difference was reported between the two acute visits. But, the VAS score show increment with the increased duration of treatment as shown in the post 2 score and as mentioned in table (4.20), which may demonstrate the increase sensation of dryness of mouth with the increase in the duration of fluoxetin therapy and is propotional to the decreased in salivary flow recorded with fluoxetine therapy.

4.4.3 Determination of xerostomia grades according to salivary flow rate

The salivary flow rate considered as an objective measure of mouth dryness and according to FR the patients arranged in to 3 grade of xerostomia were (0.1 ml/min) considered the cut off limits for hyposalivation. And as mentioned in Table (3.2), salivaryflow rate ≥ 0.2 (ml/min) is a grade 1 xerostomia, betwen (0.1– 0.2 ml/min) is grade 2 while the mas severe grade 3 in patients having salivary flow rate ≤ 0.1 (ml/min).

The numbers of patients suffer from xerostomia was recorded in each group and according to their flow rate, the grade was determined as shown in Figure (4.6). This figure clearly demonstrate that xerostomia is one of the oral adverse effects that might be caused by fluoxetine and this could be deduce from the higher numbers of observations

of diffent xerostomia grades in post1, post2 and also in the chronic patients on fluoxetin therapy.

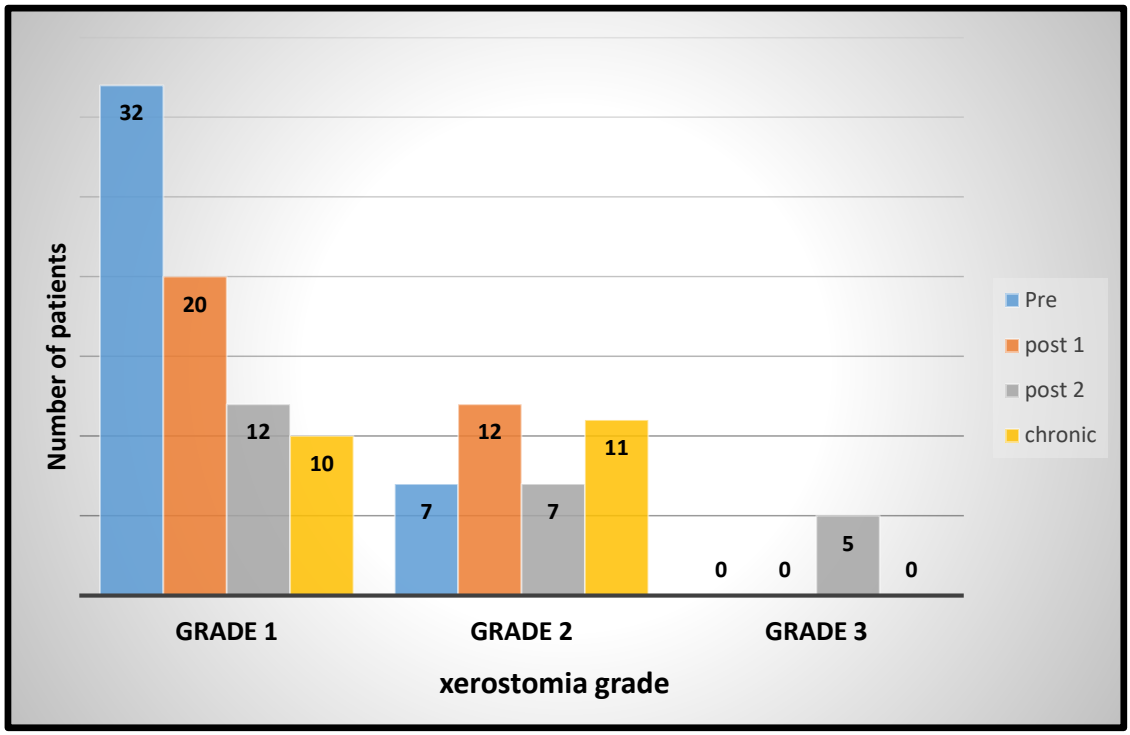

Figure 4.6: Distribution of patients according to xerostomia grades in each group.

In the pretreated level, (82%) of patients was of grade 1 xerostomia while only (18%) of grade 2. In the post 1 level, (62.5%) of the patients sufered grade 1 xerostomia with only (37.5%)o f grade 2.

In the post 2 level (50%) of the patients were included on grade 1,(29.1%) on grade 2 and (20.9%) on grade 3.

(47.6%) of the patients in the chronic group were included at grade 1 xerostomia while (52.4%) at grade 2.

4.4.4 The relation between different salivary parameters (SAA, Na^+, K^+ concentrations, FR and VAS)

Generally the relation between salivary parameters in all patients was studied to find the relations between them.

4.4.4.1 The relations betweeen different salivary parameters in the chronic group

Pearson correlation was done to determine the relation between salivary parameter in the chronic group. There were positive correlations as shown in table (4.21).

A significant positive correlation between SAA and K^+ concentrarion in the chronic group (r=0.751) and (p=0.000).

SAA also showed positive correlation with Na^+ concentration and with VAS at p>0.01, while negative correlation with FR was found at p> 0.01.

Na^+ concentration show positive correlation with SAA and K^+ concentration and with VAS score at p>0.01, while a negative correlation with FR . K^+ concentration showed negative correlation with FR at p>0.01.

Salivary FR showed significant negative correlation with VAS (r= -0.868) and (p=0.000).

Table 4.21 : The corelation between salivary parameters in the chronic group :

Salivary parameters		SAA Conc. (U/ml)	Na^+ (mmol/l)	K^+ (mmol/l)	FR (ml/min)	VAS
SAA Conc. (U/ml)	Correlation	1.000				
	Sig.	.				
Na^+ (mmol/l)	Correlation	0.190	1.000			
	Sig.	0.409	.			
K^+ (mmol/l)	Correlation	0.751**	0.021	1.000		
	Sig.	0.000	0.929	.		
FR (ml/min)	Correlation	-0.136	-0.095	-0.145	1.000	
	Sig.	0.556	0.684	0.531	.	
VAS	Correlation	0.109	0.003	0.066	-0.868	1.000
	Sig.	0.637	0.991	0.777	0.000**	.

** corelation is significant at the 0.01 level (2- tailed)

4.4.4.2 The relation between salivary parameters in the acute group

The relation between salivary parameters in the pretreated level: Pearson correlation was done between different parameters and there was a significant negative correlation between the FR and VAS (r= -0.562)(p=0.000).

Also, a negative but not significant correlation was found between FR and K^+ concentration.

SAA showed positive correlation with all parameters at p> 0.01, but K^+ concentrations showed negative correlation with FR and Na^+ concentrations at p>0.01as shown in table (4.22).

Table 4.22: The corelation between salivary parameters in the pretreated level of the acute group

Salivary parameters		SAA Conc. (U/ml)	Na^+ (mmol/l)	K^+ (mmol/l)	FR (ml/min)	VAS
SAA Conc. (U/ml)	Correlation	1.000				
	Sig.	.				
Na^+ (mmol/l)	Correlation	0.229	1.000			
	Sig.	0.160	.			
K^+ (mmol/l)	Correlation	0.081	-0.112	1.000		
	Sig.	0.632	0.498	.		
FR (ml/min)	Correlation	0.120	0.106	-0.219	1.000	
	Sig.	0.465	0.522	0.181	.	
VAS	Correlation	0.043	0.017	0.287	-0.562**	1.000
	Sig.	0.796	0.917	0.076	0.000	.

** corelation is significant at the 0.01 level (2- tailed).

The relation between salivary parameters after 4 weeks of fluoxetine therapy in the post1 level: Pearson correlation showed significant negative correlation between FR and VAS (r= -0.602)(p=0.000). Also, significant negative correlation with K^+ concentration (r= -0.499) (p=0.004) was found at the post 1 level.

VAS showed a significant positive correlation with K^+ concentration (r= 0.610) (p=0.000). SAA showed a negative correlation with VAS and positive correlation with the other parameters at p>0.01. Na^+ concentration showed negative correlation with K^+ concentration and with VAS at p>0.01. As shown in table (4.23).

The relation between salivary parameters after 8 weeks of fluoxetine therapy in the post2 level: As shown in table (4.24), Pearson correlation between salivary parameters and the FR showed a significant negative correlation with VAS (r= -0.595)(p=0.002) and also, negative correlation with SAA, Na^+ and K^+ concentrarions at p>0.01. A significant positive correlation was found between VAS and K^+ concentration (r=0.547) (p=0.006).

SAA showed only positive correlation with Na^+ concentrarion and negative correlation with other parameters at p>0.01. A negative correlation was found between Na^+ concentration and FR,VAS and K^+ concentration at p>0.01.

Table 4.23: The corelation between salivary parameters in the post 1 level of the acute group :

Salivary parameters		SAA Conc. (U/ml)	Na^+ (mmol/l)	K^+ (mmol/l)	FR (ml/min)	VAS
SAA Conc. (U/ml)	Correlation	1.000				
	Sig.	.				
Na^+ (mmol/l)	Correlation	0.110	1.000			
	Sig.	0.555	.			
K^+ (mmol/l)	Correlation	-0.223	-0.161	1.000		
	Sig.	0.227	0.387	.		
FR (ml/min)	Correlation	0.072	0.134	-0.499**	1.000	
	Sig.	0.701	0.472	0.004	.	
VAS	Correlation	-0.140	-0.009	0.610**	-0.692**	1.000
	Sig.	0.453	0.961	0.000	0.000	.

** corelation is significant at the 0.01 level (2- tailed)

Table 4.24 : The corelation between salivary parameters in the post 2 level of the acute group

Salivary parameters		SAA Conc. (U/ml)	Na^+ (mmol/l)	K^+ (mmol/l)	FR (ml/min)	VAS
SAA Conc. (U/ml)	Correlation	1.000				
	Sig.	.				
Na^+ (mmol/l)	Correlation	0.136	1.000			
	Sig.	0.525	.			
K^+ (mmol/l)	Correlation	-0.250	-0.119	1.000		
	Sig.	0.239	0.581	.		
FR (ml/min)	Correlation	-0.021	-0.100	-0.335	1.000	
	Sig.	0.924	0.643	0.109	.	
VAS	Correlation	-0.178	-0.194	0.547**	-0.595**	1.000
	Sig.	0.406	0.363	0.006	0.002	.

** corelation is significant at the 0.01 level (2- tailed)

Some of studies stated that there is a relation between SAA and salivary flow rate and that SAA activity propotional to the rate of salivary flow. Meanwhile other studies stated the contrary. Our study shows that there is no general tendency between them and this can be seen clearly in Figure 4.8.

Figure (4.8) shown that there is a negative correlation between SAA activity and FR in the chronic group and in the post 2 readings in the acute group following 8 weeks of fluoxetine administration.

Low positive correlation have been found between salivary alpha amylase activity and the salivary flow in the control group, also in pretreated and post 1 readings.

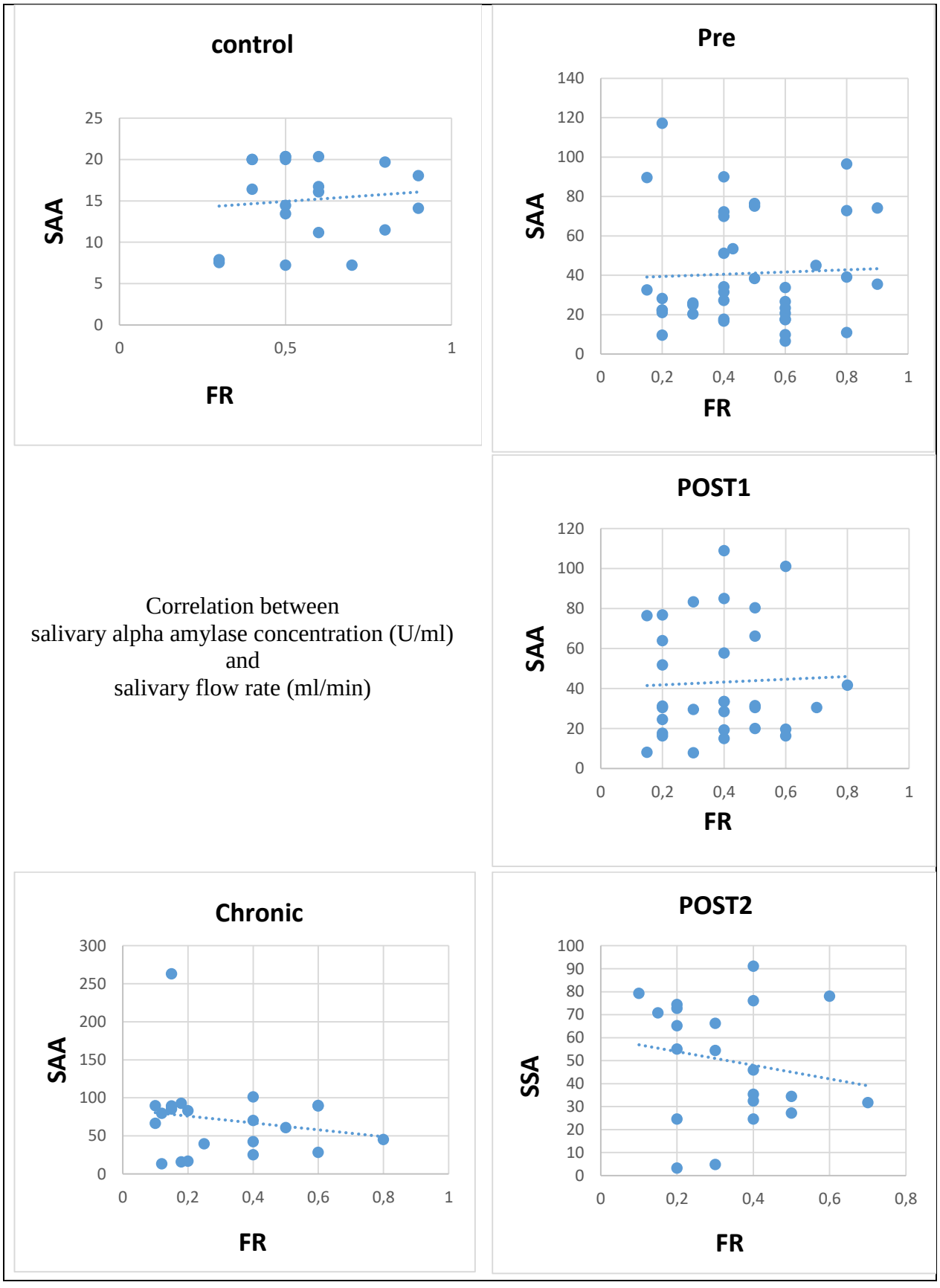

Figure 4.7: Correlation of salivary alpha amylase activity and salivary flow rate in all groups

4.5 Assesment of safety and tolerability of fluoxetine

4.5.1 Patients withdrawal

A total of (39) psychatric patients used fluoxetine in a dose ranged from (20 to 60) mg/day for a duration up to two months enrolled in this study.

Thirty nine patients were selected in follow group to be followed up to two months, from those only (23) patients complete the full acute of the study, while (16) patients (41.02%) of the total patients were withdrawn from the study due to different causes. As shown in figure (4.8). Eight patients (25.51%) withdrawn from the study due to different adverse effects caused by fluoxetine. One patientwithdrawn early within the first two weeks of the treatment due to severe TMJ problems (bruxism) which thought to be due to increased exrtrapyramidal level of serotonin caused by fluoxetine, thereby inhibiting dopamenergic pathways that control movements, also two patients (5.1%) withdrawn within the first 4 weeks of treatment due to nausea and to less extent anorexia. One patient (2.5%) claimed that he did not show any improvement of its clinical condition on fluoxetine therapy and thus he decided to stop taking drug. Four patients (10.2%) were not complaint patients (well known by the psychatric doctors that they allways not take their medications and they change it, and went from a doctor to another thinking that they might get some progress by another types of drugs).

During the next 4 weeks of the study 3 patients (7.6%) withdrawn due to moderate to severe insomnia (these patients said that they did not sleep for at least 3 to 4 continous days and even if they sleep, they would be late at 4 to 5 a.m). Another 2 men (5.1%) withdrawn from the study due to sexual adverse effects. Two patients (5.1%) were not complaint patients and they stop to have their medication after the first acute visit while another patient stop to take the drug because the drug had a low efficacy and he did not see any improvements. All these shown in table (4.25).

Table 4.25: Patients withdrawal from the study, causes and percentage.

No. of patients withdrawn from the study	%	Causes of withdrawal
8	20.51%	Adverse effects of fluoxetine
2	5.12%	Lack of efficacy
6	15.38%	Non complaint patients
16	41.02%	total

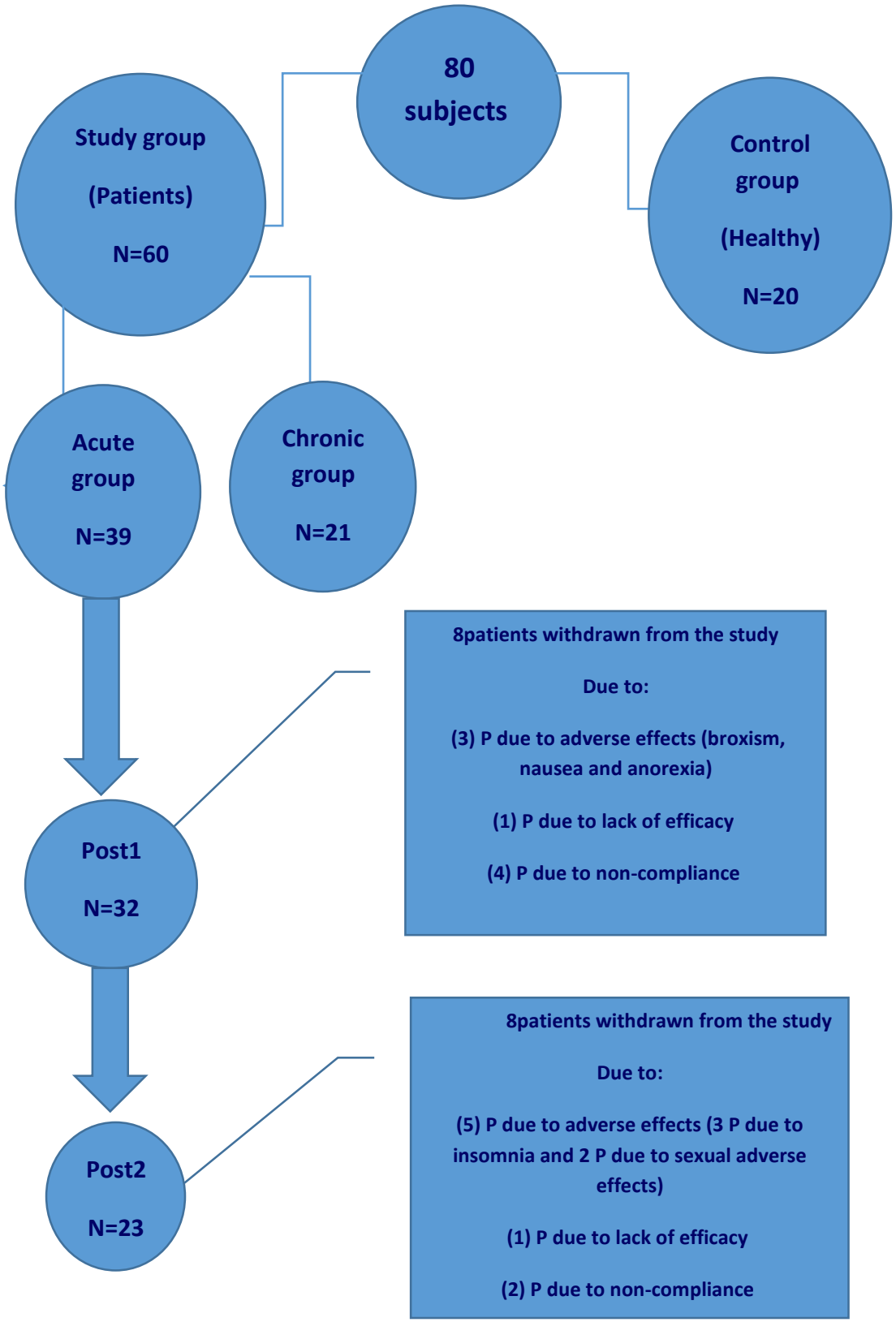

Figure (4.8): Patients withdrawal from the study. N is the numbers of subjects

Eight patients (20.51%) of the total withdrawal cases were because of adverse effects of therapy, GIT adverse effects (severe nausea and to lower extent anorexia) and TMJ problems (bruxism) occur early within the first 4 weeks of the study. While insomnia and sexual adverse effects occured within the last 4 weeks of therapy leading to discontinuation of the drug. The main adverse effects of fluoxetine that led to discontinuation of drug were shown in table (4.26).

Table 4.26: Types of adverse efffects of fluoxetine that cause withdrawal of patients from the study.

Adverse effects	No. of patients	%
G.I.T (Nausea and anorexia)	2	5.12%
insomnia	3	7.69%
Sexual adverse effects	2	5.12%
TMJ (bruxism)	1	2.56%
Total no. and %	8	20.51%

4.5.2 General adverse effects of fluoxetine

The adverse effects of fluoxetine mainly appeared in CNS system, suicidal thoughts of the patients, GIT system and on the whole body. The CNS adverse effects include: nervousness, drowsness, headache, anxiety, insomnia, tremer, abnormal dreams. GIT adverse effects include: diarrhea, constipation, nausea, anorexia, vomitting, decreased appetite.

While, the adverse effects of fluoxetine to the whole body may include: myalgia, weight loss, tachycardia, palpitation, breathing difficulties.

The incidence of many adverse effects was significantly higher in the chronic group at $p<0.05$ when compared with the control group and with the pretreated base line level of acute group. The observations on the CNS system represented the higher percentage than the GIT adverse effects, and on the body at whole as shown in figure (4.9).

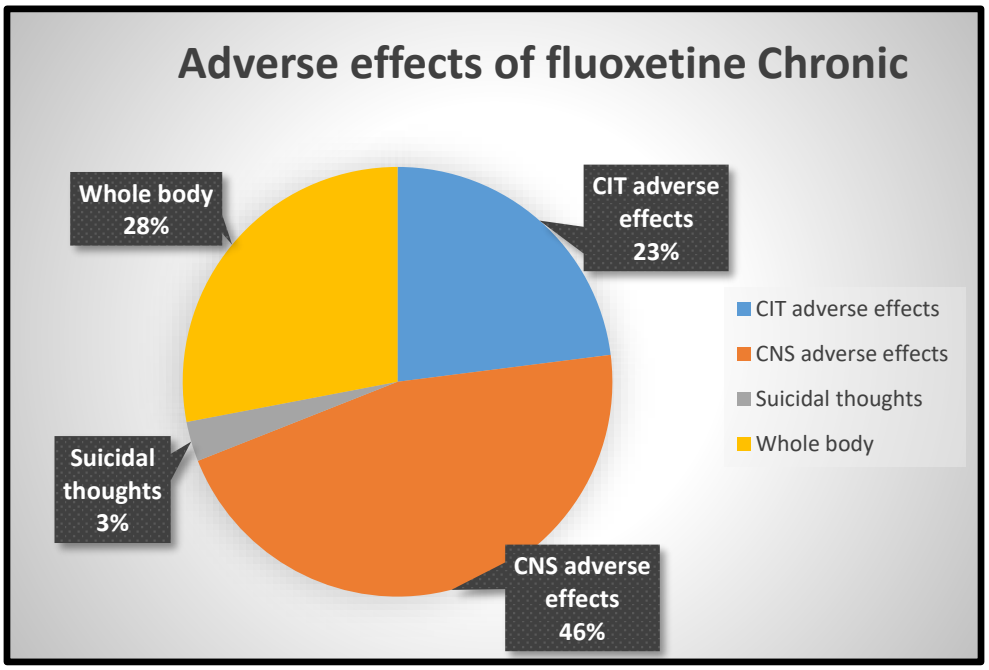

Figure 4.9: The type of adverse effects with their percentage that reported with chronic fluoxetine therapy

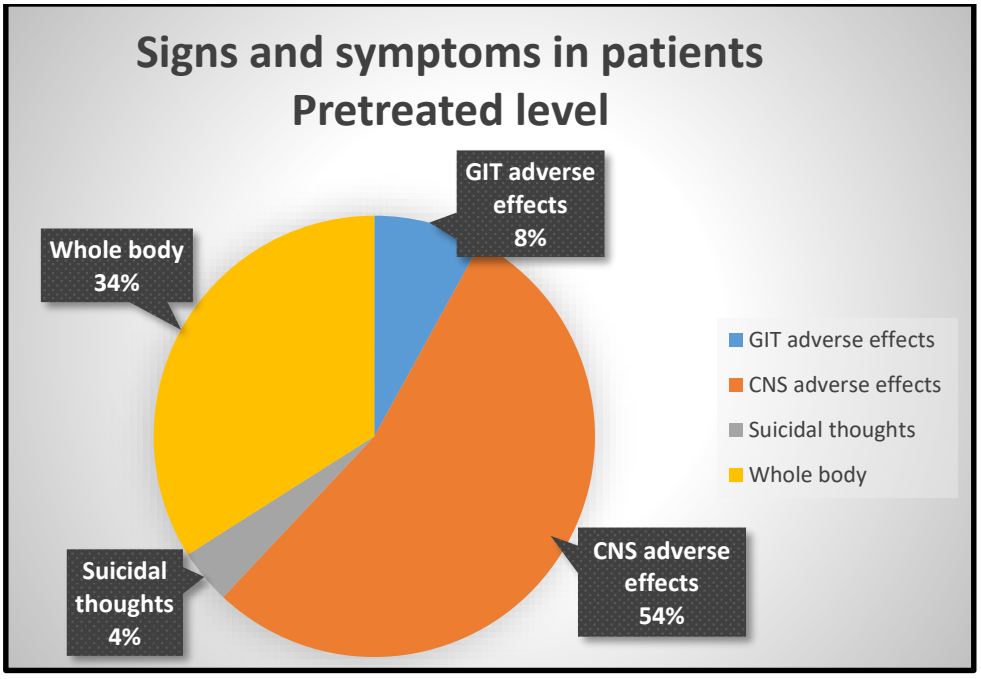

Figure 4.10: The type symptoms with their percentage that reported in the disease conditions (in the pretreated level before starting fluoxetine adminstration).

Some of these adverse effects reported higher incidence in the disease condition in the pretreated base line level before starting fluoxetine administration (might represent a symptom of the psychological condition of the patients rather than adverse effects of therapy) such as the CNS symptoms as shown in figure (4.10).

The CNS symptoms (54%) in the pretreated level, while it showed lower incidence in the chronic condition only (46%) (as an effects of improvement –due to drug therapy), while the patients compliance of GIT adverse effects were higher in the chronic condition (23%) than (16%) only in the pretreated level.

4.5.2.1 Adverse effects of fluoxetine in the chronic group

The incidence of: breathing difficulties($p=0.00$), abnormal dreams ($p=0.005$), drowsness($p=0.001$), insomnia($p=0.00$), headache($p=0.001$), anxiety($p=0.00$), weight loss($p=0.00$), decreased appetite($p=0.00$), tremor($p=0.039$), myalgia($p=0.004$), nausea($p=0.00$), anorexia($p=0.023$), tachycardia($p=0.016$) were significantly higher in the chronic group than in the pretreated level at $p<0.05$. These adverse effects were mild to moderate in severity.

While the incidence of nervousness, constipation, palpitation were higher in the base line pretreated level before starting administration of fluoxetine as an effect of disease process.

The patients complaint of many adverse effects in the chronic group can be seen in the figure (4.11).

4.5.2.2 Adverse effects of fluoxetine in the acute group

Anxiety ($p=0.00$), insomnia ($p=0.017$), nervousness($p=0.00$), breathing difficulties ($p=0.005$) all were significantly higher in the pre treated level. While, headache ($p=0.032$) were higher in post 1than in post2.

The percentge of GIT adverse effects increased with fluoxetine therapy from (34%) in post1 to (40%) in post2. While, CNS adverse effects decline from (40%) in post1to (35%) in post2 and as shown in figure (4.12).

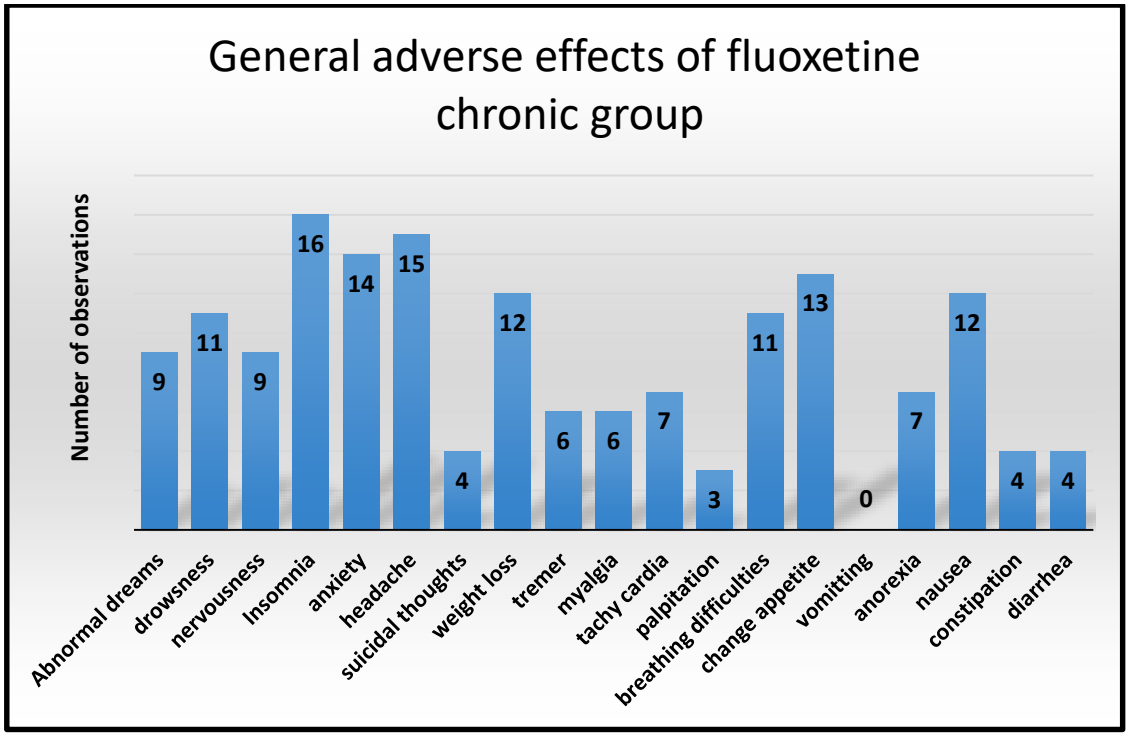

Figure 4.11: General adverse effects of fluoxetine reported in the chronic group with the number of observations.(n=21)

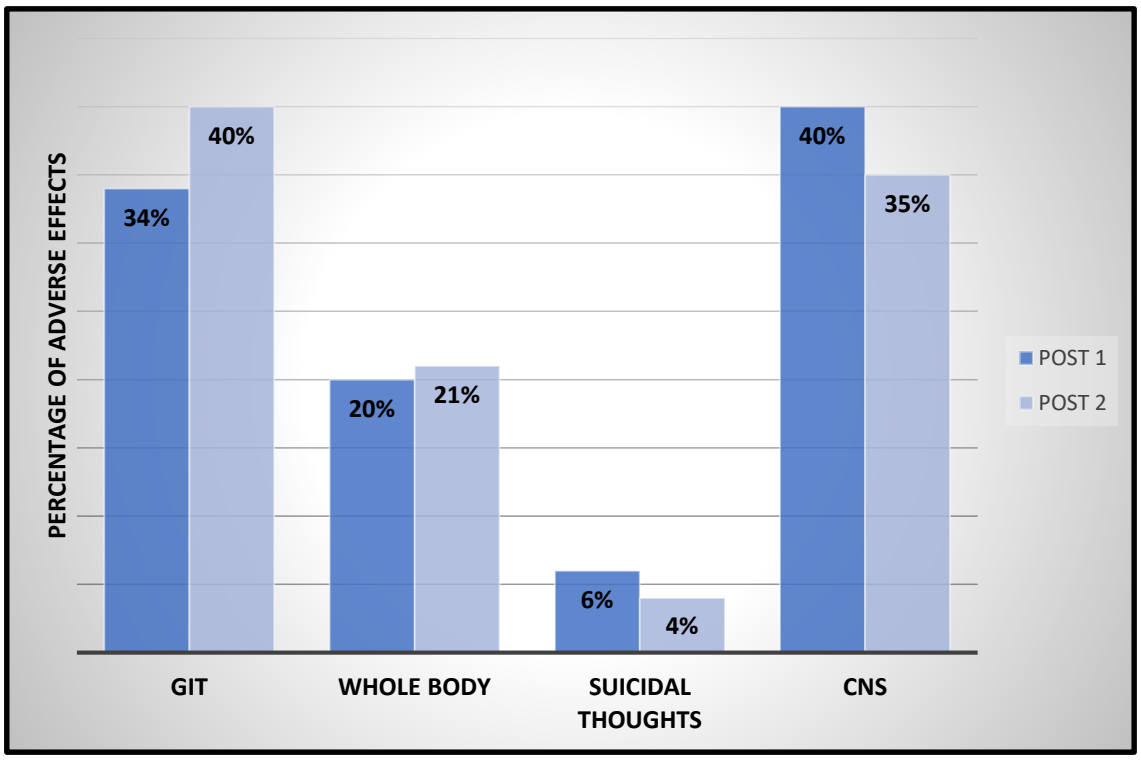

Figure 4.12: The type and percentage of adverse effects reported between post 1 and post 2 of the acute group.

Suicidal thoughts reported only in one patient (4.16%) of total (24) patients after 8 weeks of starting fluoxetine administrations.

CNS symptoms were decreased with therapy in acute group, only anxiety show number of observations in post 2 higher than post 1.

While, the patients complaints of GIT adverse effects were increased with fluoxetine therapy, as can be seen with the increased number of observations in the acute visits (post1 and 2) in group1 in this study.

Fluoxetine therapy lead to decreased patients complaints of breathing difficulties, tremer, myalgia, palpitation as improvement of the psychological condition of the pateints as effect of therapy, while it was lead to increase weight loss and myalgia and as shown in figure (4.13).

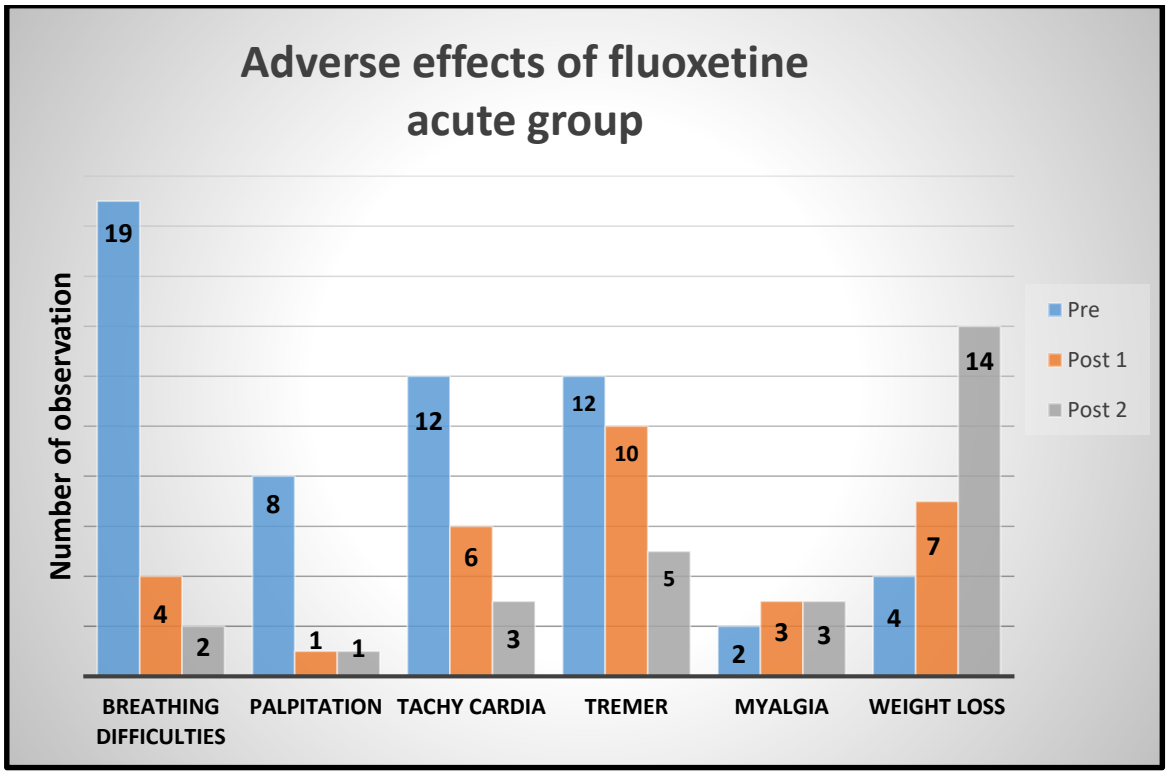

Figure 4.13 : Whole body adverse effects reported in the acute group

4.5.3 Oral adverse effects

4.5.3.1 Incidence of oral adverse effects in the chronic group

Significant higher incidence of oral adverse effects such as TMJ problems(p=0.013), glossitis(p=0.011), dysgeusia(p=0.001), xerostomia (p=0.000) were reported in the chronic group.

Patients in this group compliant mainly from xerostomia, dysguasia which appeared in 71.4%, 52.3% of the patient respectively. As shown in figure (4.14) which reported the main adverse effects caused by fluoxetine in chronic psychiatric patients.

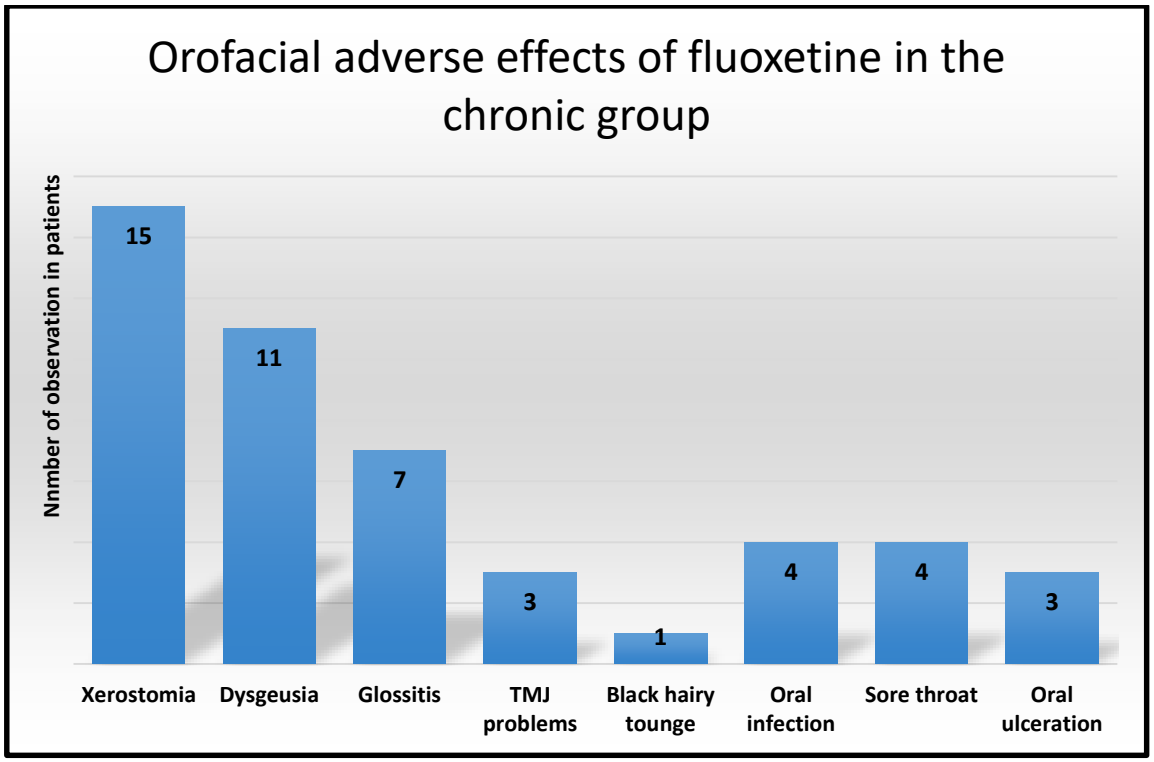

Figure 4.14 : Oral adverse effects reported in the chronic group

4.5.3.2 Oral adverse effects in the acute group

Only dysgeusia (P=0.003) and xerostomia (P=0.001) showed significant difference in this group at p value<0.05. Most of orofacial adverse effects reported increment with duration of therapy as shown in figure (4.15).

Even the increment of orofacial adverse effects reported is not significant; but, all were high in the post 2 compared with the post1 and the pretreated base line readings, while oral infections and sore throat were higher in the pre treated level than in the treated level (post 1 and 2).

(70.8%) of the patients complained of xerostomia or dry mouth in the second month followed fluoxetine therapy compared with only (43.7%) in the first month. While only (37.5%) of the patients complained from dysgeusia in the first month, this percentage rised to (54.1%) in the second month of therapy.

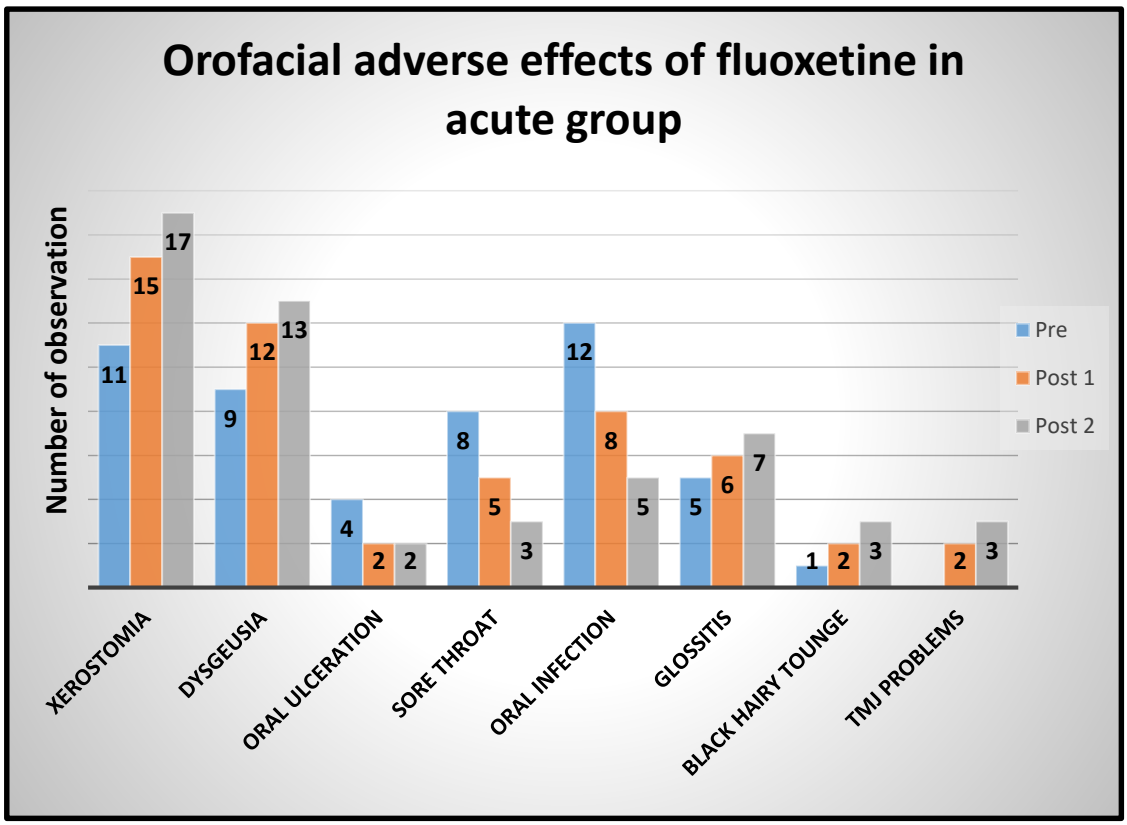

Figure 4.15: Orofacial adverse effects of fluoxetine reported in acute group

Xerostomia and dysgeusia represented the major complaint of the patients in this study as an effects of the disease process and their incedence showed increment with fluoxetine therapy in the studied groups (acute and chronic group) as an adverse effects of fluoxetine therapy, as can be shown in the following table (4.27).

Table 4.27: The incidence of major oral adverse effects in acute group and chronic group.

Groups	Nº of patients	Xerostomia %	Dysgeusia %
Pre	39	25.6	20.6
Post1	32	43.7	37.5
Post2	24	70.8	54.1
Chronic	21	71.4	52.3

4.6 Effects of gender on the biochemical changes and clinical adverse effects caused by fluoxetine therapy

4.6.1 Patients characteristics

Thirty one (51.7%) females and 29(48.3%) males were agreed to participate in this study and they distributed between groups as shown in table (4.28).

Males and females usually taking fluoxetine as a treatment of many clinical conditions and the different clinical indications of fluoxetine. In this study, the prevalance of depression was higher in females 26(43%) than males 19(31.6%).

Controversy, the prevalance of anxiety and OCD in males 8(13.3%) and 2 (3.3%) respectively were higher than females 2(3.3%) and 0(0%).

Equal prevalance of panic disorders 1(1.7%) was found between males and females in this study.

Table 4.28:The distribution of males and females between treatment groups and their percentages

Gender	Acute group no.(%)	Chronic group no.(%)	Total no.(%)
Male	22(36.7%)	7 (11.7%)	29 (48.3%)
Female	17(28.3%)	14(23.3%)	31 (51.7%)
Total no.(%)	39(65%)	21 (35%)	60 (100%)

4.6.2 Assesment of biochemical changes in saliva between males and females

4.6.2.1 Control group

Changes in SAA: Although, no significant difference reported between M and F at p value <0.05. But, the concentration of salivary amylase was higher in M (21.61± 13.33)U/ml than in F (18.92±16.57) U/ml as shown in table (4.29).

Changes in the Na^+ concentration: Despite that Na+ concentration was higher in M (50.8±7.13) than in the F (45.8±7.56) in the healthy control state. But, no significant differences was found between M and F at p value < 0.05 and as shown in table (4.27).

Changes in the K^+ concentration: No significant difference was reported between M and F in K^+ concentration at p value <0.05.

Healthy M reported lower K^+ ion concentration (37.84±17.24) than healthy F (43.86±24.84) as shown in table (4.29).

Changes in FR and and Visual Analogue Scale VAS: Although no significant difference between M and F at p value < 0.05. but, the FR was higher in M (0.63±0.18) than the F (0.5±0.14) while, the VAS score was higher in F (9.3±3.24) than M scores (8.0±2.16) as shown in table (4.29).

Generally, the highest FR reported in the healthy subject (M and F) with the lowest VAS score than the patients in followup and chronic group.

Table 4.29: The differences in salivary biochemical parameters between males and females in the control group.

Salivary parameters	Gender	mean	±Std. Deviation	Significance P value ≤0.05
SAAmylase (U/ml)	m	21.61	13.33	NS
	f	18.92	16.57	
Na^+ (mmol/l)	m	50.80	7.13	NS
	f	45.80	7.56	
K^+ (mmol/l)	m	37.84	17.24	NS
	f	43.86	24.84	
FR (ml/min)	m	0.63	0.18	NS
	f	0.5	0.14	
VAS	m	8.0	2.16	NS
	f	9.30	3.24	

4.6.2.2 Chronic group

Changes in SAA: Also, no significant difference was shown between M and F in this group at p value <0.05. but, the concentration was higher in F (56.36±33.19) than M (34.90±33.81), both concentration was higher than concentrations in the control group as shown in table (4.30).

Changes in the Na^+ concentration: Na^+ concentration was significantly high in female patients (56.154^+-7.33) than in male patients (48.29^+-9.19) at p value<0.05. Both

concentrations were higher than the concentration in the control group as shown in table (4.30).

Changes in the K^+ concentration: Chronic male patient as healthy males, showed K^+ ion concentration (33.82±18.20) lower than chronic females patient (34.87±21.56). Both concentration lower than in the control group.

Changes in FR and and visual analogue scale VAS: FR in male patients was higher (0.34±0.21) than female patients (0.32±0.23); while the females show higher VAS score (29±8.87) than the males (28.29±9.30). No significant differences reported in this group at p value ≤0.05.

The salivary flow rate of males and females in this group were lower than the healthy males and females in the control group while VAS was controversy with this.

Table 4.30: The differences in salivary parameters between males and females in the chronic group.

Salivary parameters	gender	mean	±Std. Deviation	Significance P value ≤0.05
SAAmylase (U/ml)	m	34.90857	33.812042	NS
	f	56.36114	33.191361	
Na^+ (mmol/l)	m	48.29	9.196	0.047
	f	56.14	7.336	
K^+ (mmol/l)	m	33.829	18.2002	NS
	f	34.871	21.5637	
FR (ml/min)	m	.34386	.214750	NS
	f	.32143	.234582	
VAS	m	28.29	9.30	NS
	f	29	8.87	

4.6.2.3 Acute group

Changes in SAA: Also, no significant difference reported at p value < 0.05. But, as in the chronic group, the concentration was higher in female (57.64±60.82) than male (36.0±24.46), as shown in table (4.31). These concentrations were higher than the chronic and the control group

Changes in the Na^+ concentration: No significant effect of gender on Na^+ concentration was found in this group. But generally, the concentration of males (50.55±7.36) was slightly higher than females concentration (50.47±7.56), as shown in table (4.31).

Changes in the K^+ concentration: Although, no significant difference reported in this group between male and female. But, females showed higher K^+ion concentration (46.85±30.88) than males (33.70±20.86). Females in this group reported higher concentration than chronic female patients and than the healthy femalesas shown in table (4.31).

Changes in FR and in visual analogue scale VAS: No significant difference reported in this group between male and female at p value ≤0.05. FR in male patients was higher (0.51±0.24) than female patients (0.39±0.15) while the female show higher VAS score (18±8.41) than the male group (17.5±7.9). The salivary flow rate of male and female in this group were lower than the healthy male and female in the control group while VAS was contraversy with this.

Table 4.31: The differences in salivary parameters between males and females in the acute group.

Salivary parameters	gender	mean	±Std. Deviation	Significance P value ≤0.05
Amylase (U/ml)	m	36.00400	24.46	NS
	f	57.64471	60.82	
Na^+ (mmol/l)	m	50.55	7.360	NS
	f	50.47	7.567	
K^+ (mmol/l)	m	33.700	20.86	NS
	f	46.859	30.88	
FR (ml/min)	m	.50682	.2479	NS
	f	.40806	.1521	
VAS	m	17.59	7.938	NS
	f	17.41	8.419	

4.6.3 Effect of gender on safety and tolerability of fluoxetine

4.6.3.1 Fluoxetine withdrawal

Nine males (56.25% of the total withdrawal cases) were withdrawn from this study for different reasons, which was higher than the females withdrawal percentage (only 7 females withdrawn representing 43.75%).

Also, females was more complaint than male which show higher withdrawal rate due to not compliance (25%) than only (12.5%) in females as shown in table (4.30).

The first patient withdrawn in this study was a female, early within the first two weeks, due to severe nausea caused by fluoxetine, followed by a male who stop taking fluoxetine due to TMJ problems and orofacial pain, occur 2 weeks after starting the administration of fluoxetine as he claimed, this patients refused to expose to physical examination to diagnose his condition as shown in table (4.32).

Table 4.32: Causes of withdrawal of males and females.

Gender	GIT	insomnia	TMJ bruxism	Sexual adverse effects	Lack of efficacy	Not compliance	Total no (%)
M	0 (0%)	1 (6.25%)	1 (6.25%)	2 (12.5%)	1 (6.25%)	4 (25%)	9 (56.25%
F	2 (12.5%)	2 (12.5%)	0 (0%)	0 (0%)	1 (6.25%)	2 (12.5%)	7 (43.75%)
total	2 (12.5%)	3 (18.75%)	1 (6.25%)	2 (12.5%)	2 (12.5%)	6 (37.5%)	16 (100%)

4.6.3.2 Assesment of general adverse effects of fluoxetine

Chronic group: Anxiety ($p=0.038$), headache ($p=0.04$), nervousness ($p=0.042$) were significantly higher incidence in M than F at $p<0.05$.

Acute group: No significant difference was shown in the pre treated level and post 1 between M and F at p value <0.05, but in post 2 group constipation ($p=0.048$), palpitation ($p=0.046$), anxiety ($p=0.04$), nervouusness ($p=0.032$) were significant higher in M than

F. Although the change were not significant but nausea, myalgia, insomnia were higher in F than M.

4.6.3.3 Assesment of orofacial adverse effects of fluoxetine

Chronic group: Only taste change (dysgeusia) was significantly higher in M than F at (P=0.035).

Acute group: TMJ problems (bruxism), dysgeusia were significantly higher in M than F at (p=0.04, p=0.035) respectively.

4.7 Effects of different doses of fluoxetine on salivary flow and content and on the incidence of general and oral adverse effects on psychaitric patients

The patients were classified according to the dose into 3 groups:

- Group1: including patients taking fluoxetine in adose of 20mg/d.
- Group2: including patients taking fluoxetine in adose of 40mg/d.
- Group3: including patients taking fluoxetine in adose of 60mg/d.

4.7.1 Chronic group

The patients enrolled in this group have different doses of fluoxetine and as shown in table (4.33).

Table 4.33: The distribution of different doses of fluoxetine in the chronic group

Dose mg/d	No of patients
20 mg/d	8
40 mg/d	13
60 mg/d	0
Total number	21

From all the biochemical parameters in saliva which have been studied in this group, only Na^+ concentration showed significant differences with dose at p value≤0.05. Itwas higher in patients taking fluoxetine 40 mg/d (54.62±7.93) than in patient taking 20 mg/d of fluoxetine (46.0±7.5). FR and VAS showed no significant differences with different dose of fluoxetine, as shown in table (4.34).

From general adverse effects reported with fluoxetine, only nervousness was highly significant in patients on dose (40 mg/d) fluoxetine than in (20 mg/d) at p =0.035.

Although no significant difference was shown but, most of the general adverse effects of fluoxetine showed slight increase in appearance with increased dose, such as weight loss, change appetite, tremer, myalgia, nausea, anorexia, suicidal thoughts, nervousness, anxiety and insomnia.

No significant difference reported in orofacial adverse effects. But, their mean rank of oral infection, dysgeusia and/or xerostomia were increased with the dose increased.

Table 4.34: Differences in salivary parameters in different fluoxetine doses in the chronic group

Salivary parameters	Dose (mg/d)	NO.	Mean±SD	significance
SAA(U/ml)	20	8	50.31±36.48	NS
	40	13	83.72±58.53	
Na^+ (mmol/l)	20	8	46.00±7.56	0.024
	40	13	54.62±7.932	
K^+(mmol/l)	20	8	46.60±17.31	NS
	40	13	46.34±29.28	
FR(ml/min)	20	8	0.319±0.18	NS
	40	13	0.34±0.25	
VAS	20	8	27.75±16.03	NS
	40	13	29.85±13.20	

4.7.2 Acute group

All the (39) patients in this group took fluoxetine capsule (20 mg/d) as a starting dose, so all the patients in post1 was in this dose. But in post 2, the patients who completed the study have different doses of fluoxetine according to its medical state and response to fluoxetine therapy and as shown in table (4.35).

Table 4.35 : The distribution of different doses of fluoxetine in the acute group

Dose mg/d	No of patients
20mg/d	11
40mg/d	9
60 mg/d	4
Total number	24

Generally, no significant difference were reported in the salivary parameters in relation to dose of fluoxetine as shown in table (4.36).

Although no significant difference was shown but, most of the general adverse effects of fluoxetine showed slight increase in appearance with increased dose, and from the general adverse effects caused by fluoxetine, only anxiety reported higher significant in dose (60mg/d) than other doses at (p value = 0.035).

While no significant oral adverse effects reported in this study in relation to dose.

Table 4.36 : Differences in salivary parameters in different fluoxetine doses in the second month of therapy

Salivary parameters	Dose (mg/d)	NO.	Mean±SD	significance
Amylase	20	11	52.43±33.19	NS
	40	9	54.96±33.81	
	60	4	55.84±24.46	
Na^+	20	11	43.69±7.57	NS
	40	9	45.86±9.20	
	60	4	49.50±7.36	
K^+	20	11	41.77±21.56	NS
	40	9	46.88±18.20	
	60	4	49.45±20,87	
FR	20	11	0.29±0.25	NS
	40	9	0.31±0.15	
	60	4	0.40±0,23	
VAS	20	11	21.50±8,42	NS
	40	9	30.50±7.94	
	60	4	32.85±13.87	

4.8 Effects of different durations of fluoxetine therapy on salivary flow and contents and on the general and oral adverse effects

The patients arranged into four groups according to durations of fluoxetine therapy and as follows in table (4.37). Although the number of patients was small, but we try to find the differences in the salivary parameters that might caused by fluoxetine therapy chronicly and which types of adverse effects that might happen in relation to duration of fluoxetine therapy.

Table 4.37: The distribution of patients in groups according to duration of fluoxetine therapy

Group	Duration of therapy (months)	No of patients
Group 1	Two months	28
Group 2	More than 2 and less than 4	13
Group 3	More than 4 and less than 6	3
Group 4	More than 6 month	2
Total number		46

4.8.1 Biochemical changes in saliva

SAA and Na^+ ion and K^+ ion concentrations: One way Anova and Duncan Tests showed that all these salivary parameters had no significant difference between the four group at $p < 0.05$.

Salivary Flow Rate: One way Anova showed significant difference between these groups at p=0.015. Duncan test showed that there was a significant difference between group 3 and the pretreated base line level at p value ≤0.05, which reported the highest FR (0.46 ml/min), while group 3 represent the lowest FR (0.16 ml/min). No significant difference of FR of group 1 and 2 with both the pre treated level and group 3. But, group 1 showed higher FR (0.34 ml/min) than group 2.

VAS: One way Anova showed significant difference between these groups at p=0.0001 Although group 1 and the pretreated base line level showed no significant difference, but group 1 showed higher VAS score (27.23±9.8) than the base line score (17.51±11.02). These both groups were significantly lower than group 2 (30.38±5.8) and

group 3 which higher VAS score than all groups (40.50±6.2) at p value ≤0.05. While no significance between group 2 and 3 at p≤0.05.

4.8.2 Assesment of safety and tolerability of fluoxetine

Kruskal–wallis Test and chi-square showed some significant differences in the general and orofacial adverse effects in relation to duration of fluoxetine therapy.

4.8.2.1 General adverse effects

Weight loss (p=0.041), myalgia (p=0.043), nausea (p=0.000), breathing diffculties (p=0.007), nervousness (p=0.000), insomnia (p=0.005), constipation (p=0,048) and anxiety (p=0.000); all showed significant differences with incresed duration of fluoxetine therapy at p ≤0.05.

Weight loss, myalgia, nausea were increased with duration in group 1 and 2. While breathing difficulties, nervousness, insomnia and anxiety appeare to be high in the pretreated level (as an effect of disease) and they were decreased in the first group (due to the short term effect of fluoxetine therapy), and they increased with increase duration of therapy (high in group 2 and 3).

4.8.2.2 Oral adverse effects

TMJ problems (p=0.002), black hairy toungue (p=0.027) were significantly high in group 1 and 4; dysgeusia (p=0.04) were significantly high in group 3 and 4; and xerostomia (p=0.01) were significantly high with increased duration of fluoxetine therapy at p≤0.05 in group 2 and 3.

4.9 Determination of serum fluoxetine level by high performance liquid chromatography (HPLC) and their relation to other patients' data

All patients being treated with antidepressants for any indication should be monitored appropriately and observed closely for clinical worsening, suicidality, and unusual changes in behavior, especially during the initial few months of a course of drug therapy, or at times of dose changes, either increases or decreases.

Serum concentration measurement for fluoxetine was done for the sixty patients enrolled in this study as can be seen in figure (4.16). In general, the mean concentration was higher in females than males in the chronic and acute group.

A wide range of fluoxetine concentration have been reported in the study in relation to dose.

No clear relation noticed between drug concentration and a patients' withdrawal from the study, and between concentration and adverse effect occurred in the patient.

Direct relation was found between the fluoxetine concentration and oral adverse effects (xerostomia); the incidence of xerostomia was increased in the higher drug concentrations.

Salivary flow rate showed decrement with the higher fluoxetine concentrations. SAA activity show indirect relation with the concentration in some readings.

The concentration of Na+ ion show a pattren of declinment with the low fluoxetine concentration while this pattren was loosed at the higher concentration of fluoxetine which make it difficult to conclude the relation between them. This relation was more clear with potassium ion concentration which report indirect relations with lower fluoxetine concentrations than higher concentration.

The pattren of relations that have been reported between fluoxetine conc. and the concentrations of Na+ and K+ ions and SAA activity in the lower drug concentration and lower doses give an expresion the activity of fluoxetine might be better low dose than in high dose.

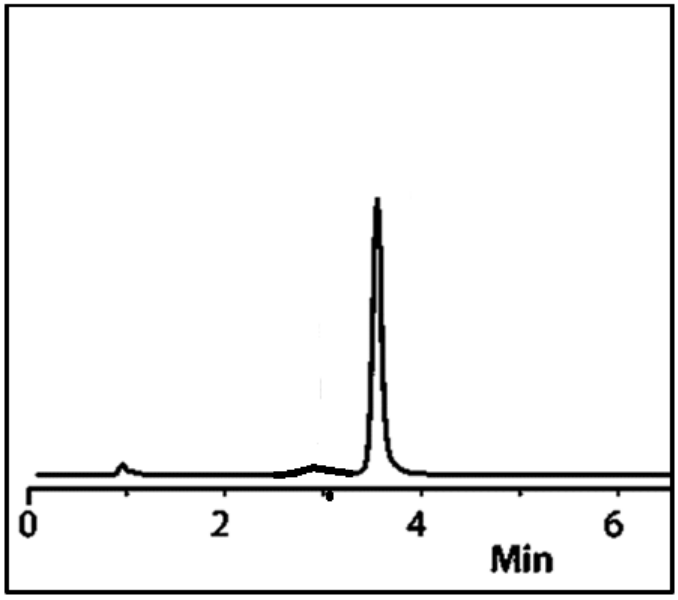

Figure 4.16: fluoxetine HPLC chromatogram with the chromatographic condition was as follows: retention time 3min, flow rate 1.5ml/min, injection volume 0.1ml, temperature 22C°.

4.9.1 Measurements of fluoxetine levels in the chronic group

The relatively slow elimination of fluoxetine (elimination half-life of 1 to 3 days after acute administration and 4 to 6 days after chronic administration) and its active metabolite, nor-fluoxetine (elimination half-life of 4 to 16 days after acute and chronic administration), leads to significant accumulation of these active species in chronic use and delayed attainment of steady state, even when a fixed dose is used.

As mentioned previously in Table 4.31, eight of the patients in the chronic group was taken fluoxetine in a dose 20 mg/day and thirteen patients was taken fluoxetine 40 mg/day. The mean serum fluoxetine concentration in patients on dose 20 mg/d fluoxetine therapy was (87.51±41.83) µg/L and C max. was 142.55 µg/L.The mean serum fluoxetine concentration in females on dose 20 mg/d fluoxetine therapy was (99.75±30.37) µg/L while (64.42±23.61) µg/L.

Although no significant changes was found between the serum fluoxetine concentration and salivary parameters (SAA and Na^+ ion and K^+ ion concentrations) but, there is a slight inverse linear relation ship between SAA level and fluoxetinne concentration which can be notice from Figure (4.17).

The SAA level was low with the higher fluoxetine concentrations. Also indirect relation can be notice between the concentration of drug and Na^+ ion and K^+ ion concentracion.

The patients in general having salivary flow rate more than those taking fluoxetine 40mg/d but no relation founded between drug concentration and FR and between drug concentration and VAS.

The mean serum fluoxetine concentration in patients on dose 40 mg/d fluoxetine therapy was (108.59±54.17) µg/L and C max. was 171.95 µg/L.

The mean serum fluoxetine concentration in females on dose 40 mg/d fluoxetine therapy was (108.32±34.81) µg/L while for males it was (96.36±45.35) µg/L.

No significant changes found between salivary parameters and drug concentration in patients with a dose 40 mg/d. SAA level was higher in the patients with low fluoxetine level and was decreased in the higher levels and there is indirect relation between them can be noticed clearly in figure (4.18). Also we can notice an inverse relation with Na+ ion and K+ ion concentrations, which might indicate the effect of the drug on the psychological state of the patients.

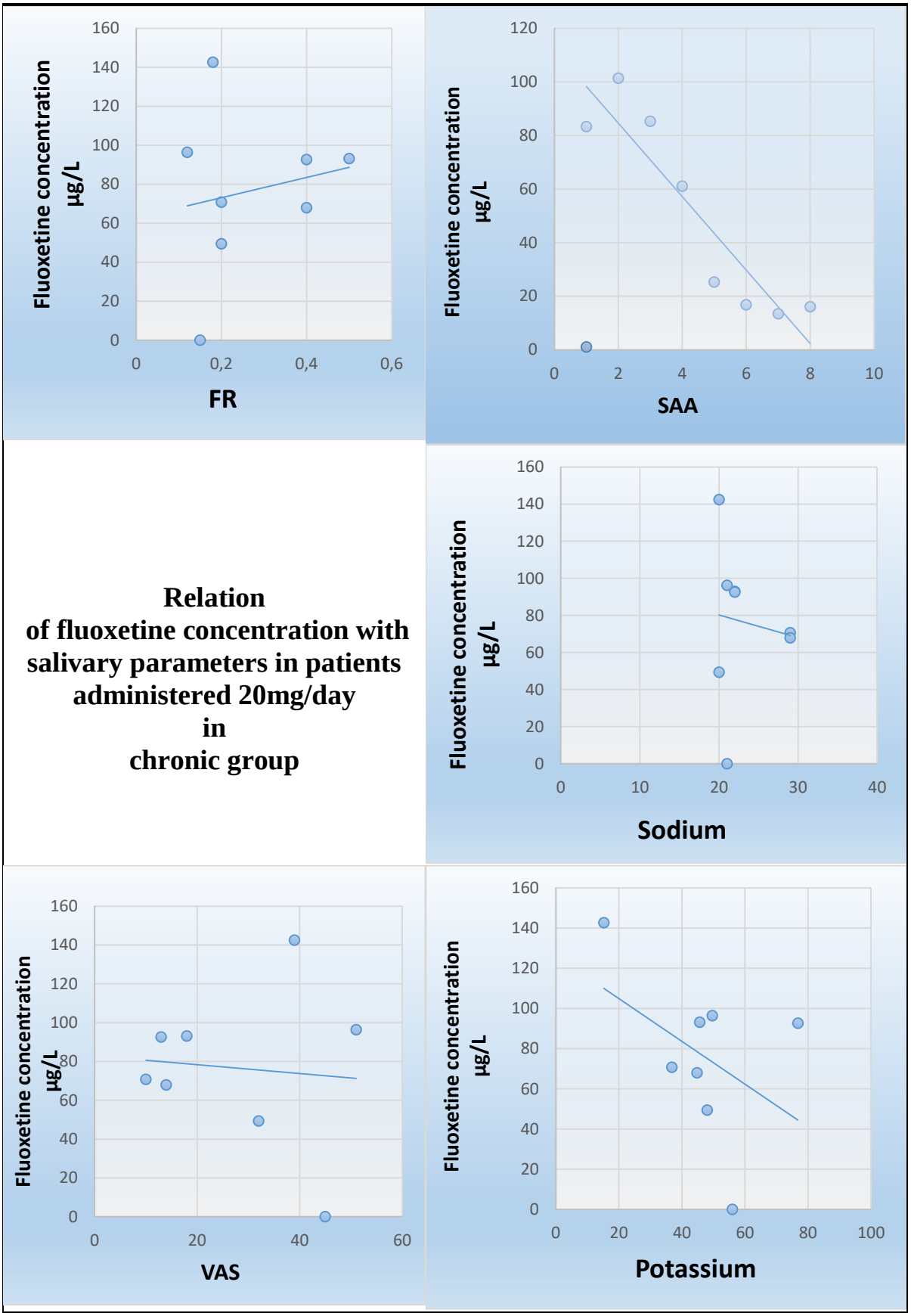

Figure 4.17: The relation between fluoxetine concentration and salivary parameters in the chronic group at dose 20mg/day.

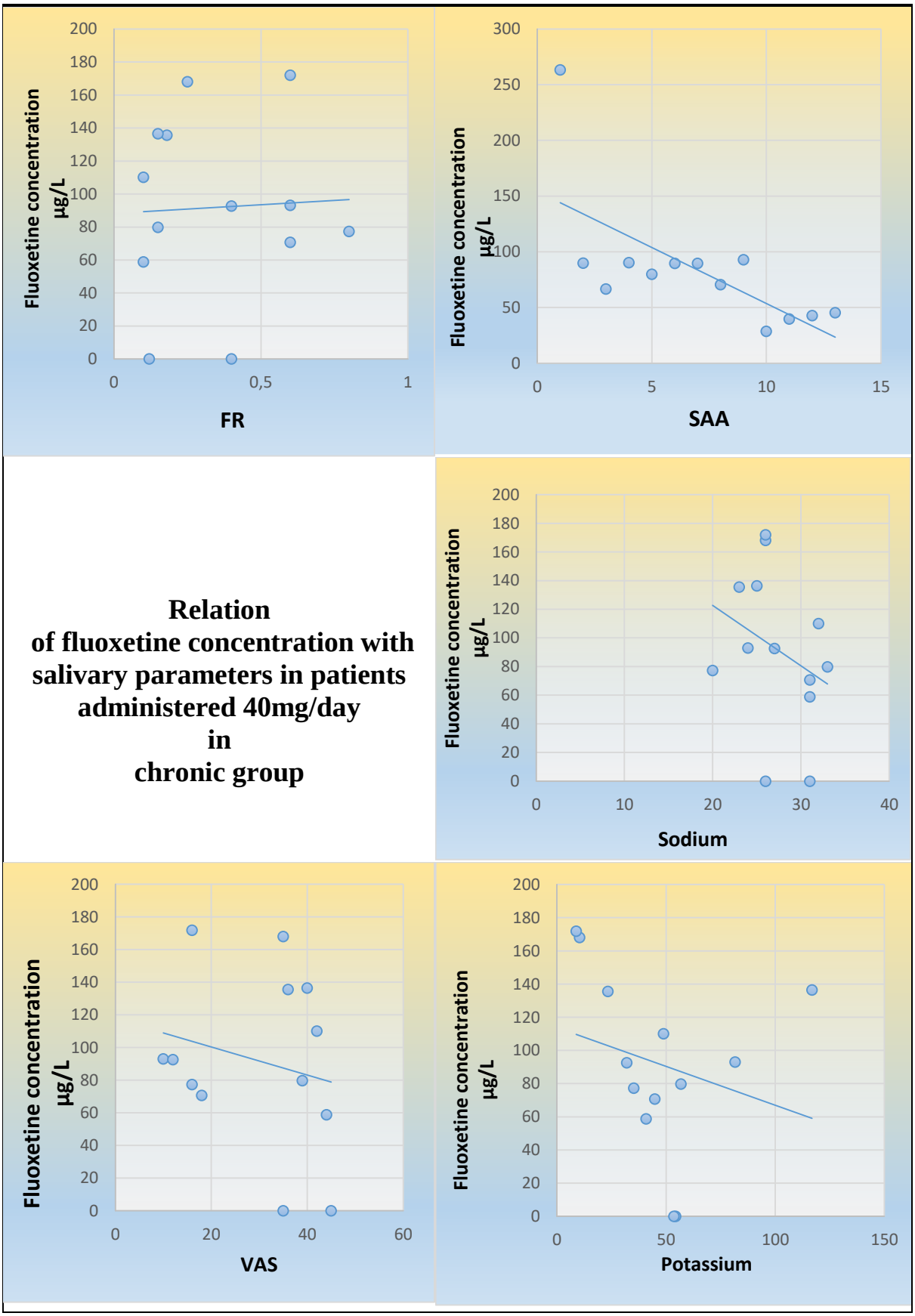

Figure 4.18: The relation between fluoxetine concentration and salivary parameters in the chronic group at dose 40 mg/day.

4.9.2 Measurements of fluoxetine levels in the acute group

Thirty-nine patients with different psychological disorders have been selected to participate in this study and have been followed for 8 weeks of fluoxetine therapy. The patients have been examined and diagnosed by the physician and selected to participated in this part of the study if they were physiologically well and without any acute illness or any liver or renal problems. The study aim to report the effects of fluoxetine on the patients and excluding any other factors that may interfere with the investigation.

Two of these patients have been detected a level of fluoxetine in blood in the first reading (before starting therapy) in spite of a two weeks of wash out that have been applied for all patients before starting fluoxetine administration, thus their reading was excluded. All the patient readings were within the therapeutic level of dosing 20-60 mg/d.

4.9.2.1 Measurements of fluoxetine levels in the acute group, Post1

In this group all patients starting with a dose 20mg/d, thus all patients' readings in post1(after one month of fluoxetine therapy) was in this dose. And the mean serum fluoxetine concentration in patients on dose 20mg/d fluoxetine therapy was (97.31±33.58) µg/L and C max. was 168,85 µg/L.

The mean serum fluoxetine concentration in females on dose 20mg/d fluoxetine therapy after one month of therapy was (104.90±36.48) µg/L while it was (89.71±27.87) µg/L in males. Although no significant changes was found between the serum fluoxetine concentration and salivary parameters (SAA and Na^+ ion and K^+ ion concentrations) but, there is a inverse linear relation ship between SAA level and fluoxetinne concentration which can be notice from Figure (4.19).

The SAA activity was low with the higher fluoxetine concentrations and was high in patients with low therapeutic fluoxetine concentration. Also indirect relation can be notice between the concentration of drug and K^+ ion concentration. While no relation recorded with Na^+ ion concentracion, FR, and VAS.

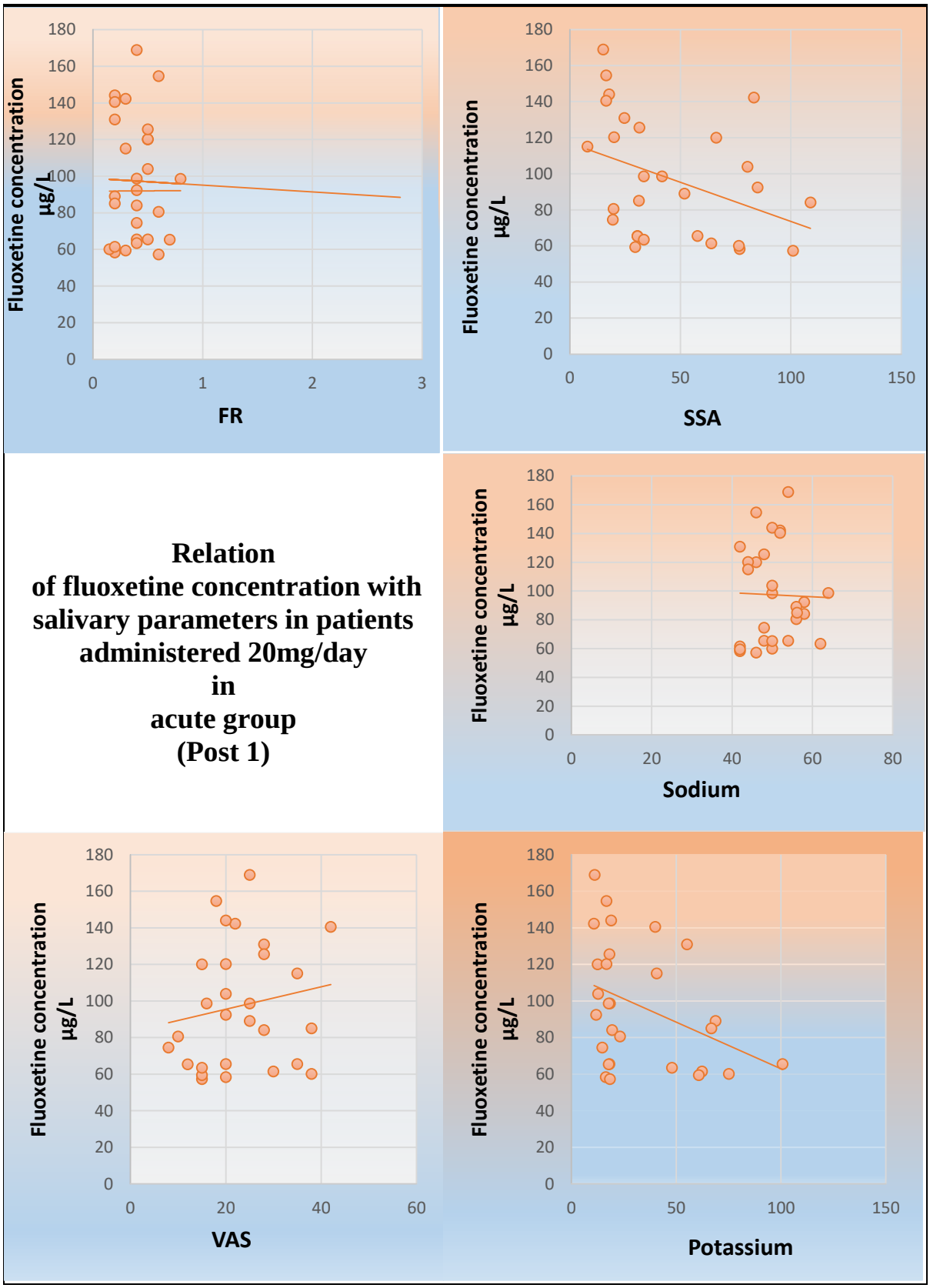

Figure 4.19: The relation between fluoxetine concentration and salivary parameters in the acute group at dose 20 mg/day, post1 after one month of therapy.

4.9.2.2 Measurements of fluoxetine levels in the acute group, Post2

In the second month of therapy, the patients were having different dose according to their response and their psychological situations. Thus the dose of fluoxetine was 20-60 mg/d.

The mean serum fluoxetine concentration in patients on dose 20 mg/d in the second month of fluoxetine therapy in the acute group was (128,88± 31,81) µg/L and C max. was 174,62 µg/L.

Females show a little higher fluoxetine level on dose 20 mg/d fluoxetine therapy after two months of therapy (131.90±40.83) µg/L than male level which was (125.85±22.43) µg/L.

No significant changes found between salivary parameters and drug concentration in patients with a dose 20mg/d.

No clear relation found between concentration and SAA activity or with Na^+ ion and K^+ ion concentracion as can be notice in figure (4.20).

While the mean serum fluoxetine concentration in patients on dose 40 mg/d in the second month of fluoxetine therapy in the acute group was (172.85± 33.78) µg/L and C max. was 240.2 µg/L.

Also females show a little higher fluoxetin level on dose 40 mg/d fluoxetine therapy after two months of therapy (175.32±42.09) µg/L than males level which was (170.37±27.94) µg/L.

No significant changes were founded between salivary parameters and drug concentration in patients with a dose 40 mg/d. The salivary flow rate was minimum than in patients with 20 mg/d fluoxetine and decreased with the increment of drug concentration.

The VAS was higher and showed a direct relation with the concentration of fluoxetine measured while no clear relation was founded between concentration and SAA activity or with K^+ ion concentracion. Na^+ concentration show increment with the increased drug concentration as can be noticed in figure (4.21).

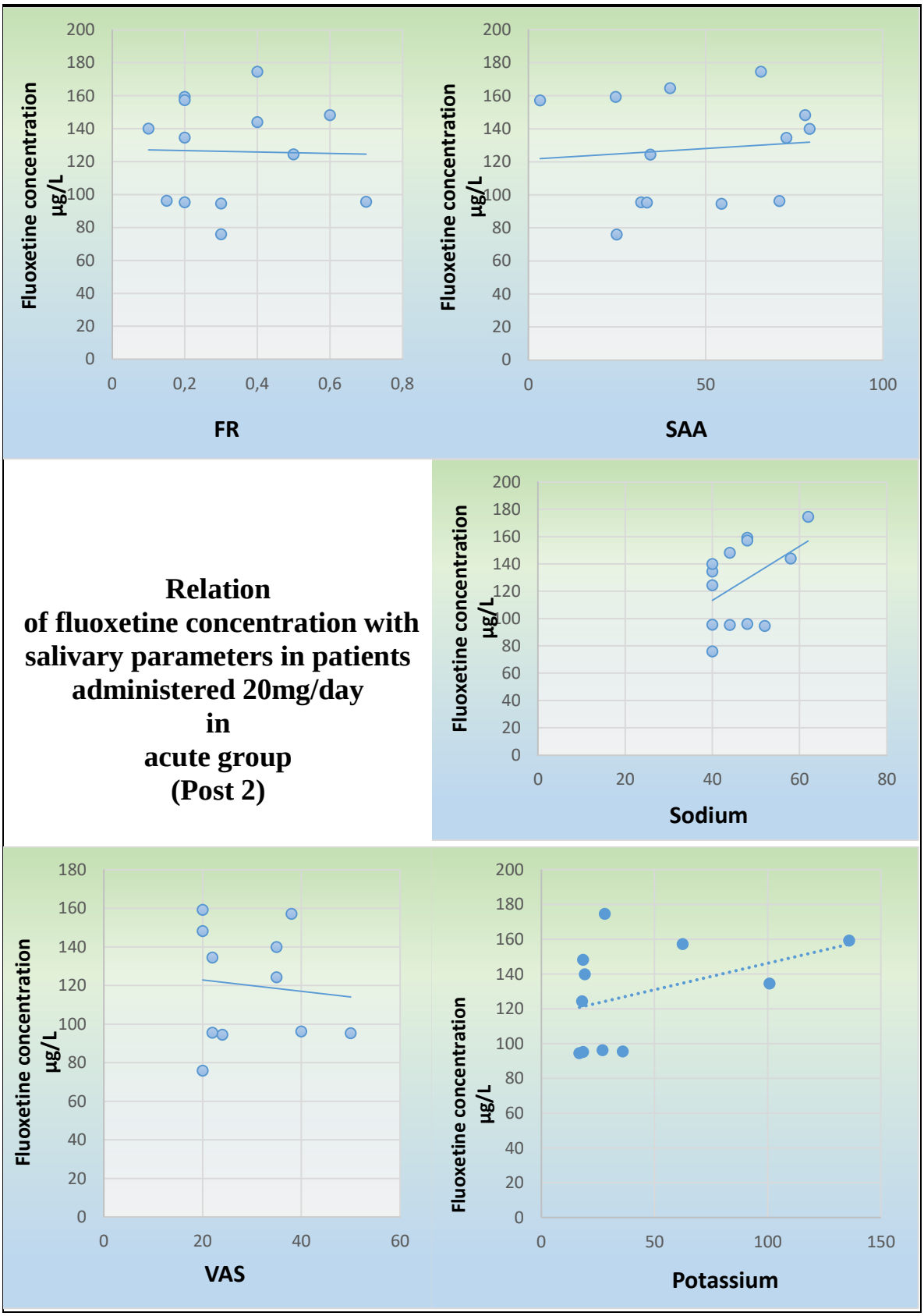

Figure 4.20: The relation between fluoxetine concentration and salivary parameters in the acute group at dose 20 mg/day, post2 after two months of therapy.

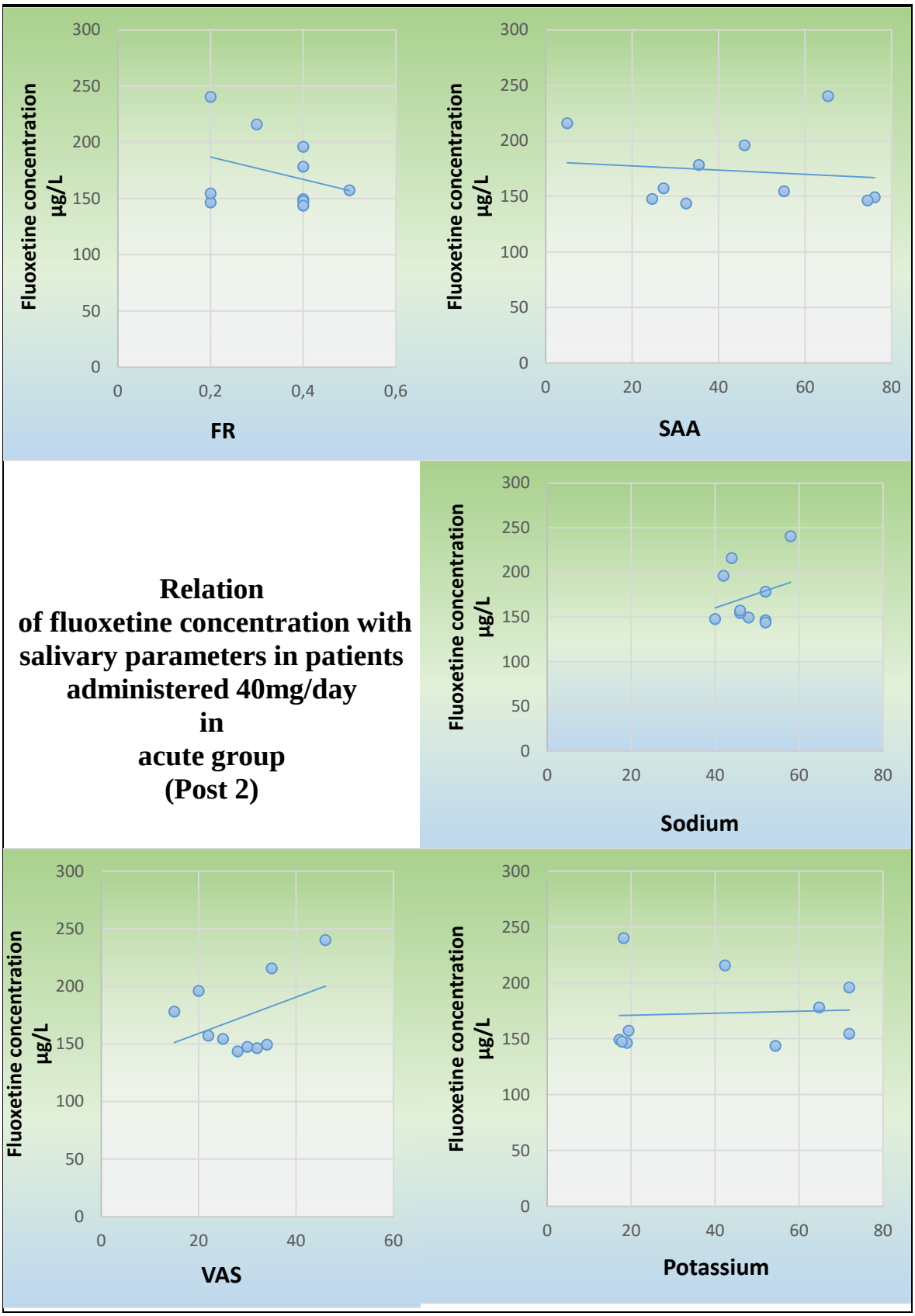

Figure 4.21: The relation between fluoxetine concentration and salivary parameters in the acute group at dose 40 mg/day, post2 after two months of therapy.

At dose 60 mg/d of fluoxetine, the FR was low with the high fluoxetine concentration. A clear indirect relationship was noticed and a negative effect of fluoxetine on the salivary flow can be noticed clearly in figure (4.22).

The salivary flow rate decreased when fluoxetine dose and serum concentration increased. Also indirect relations was reported with VAS.

Direct proportional relation have been reported between drug concentration and Na^+ and while no cler relation reported with K^+ concentrations.

No clear relation reported in this dose with the SAA activity. However the number of patients was small.

The mean serum fluoxetine concentration in patients on dose 60 mg/d in the second month of fluoxetine therapy in the acute group was (283.69± 40.73) µg/L and C max. was (331.16 µg/L).

Females show a higher serum fluoxetin level on dose 20 mg/d fluoxetine therapy after two months of therapy (307.39±33.61) µg/L than male level which was (259.95±40.02) µg/L.

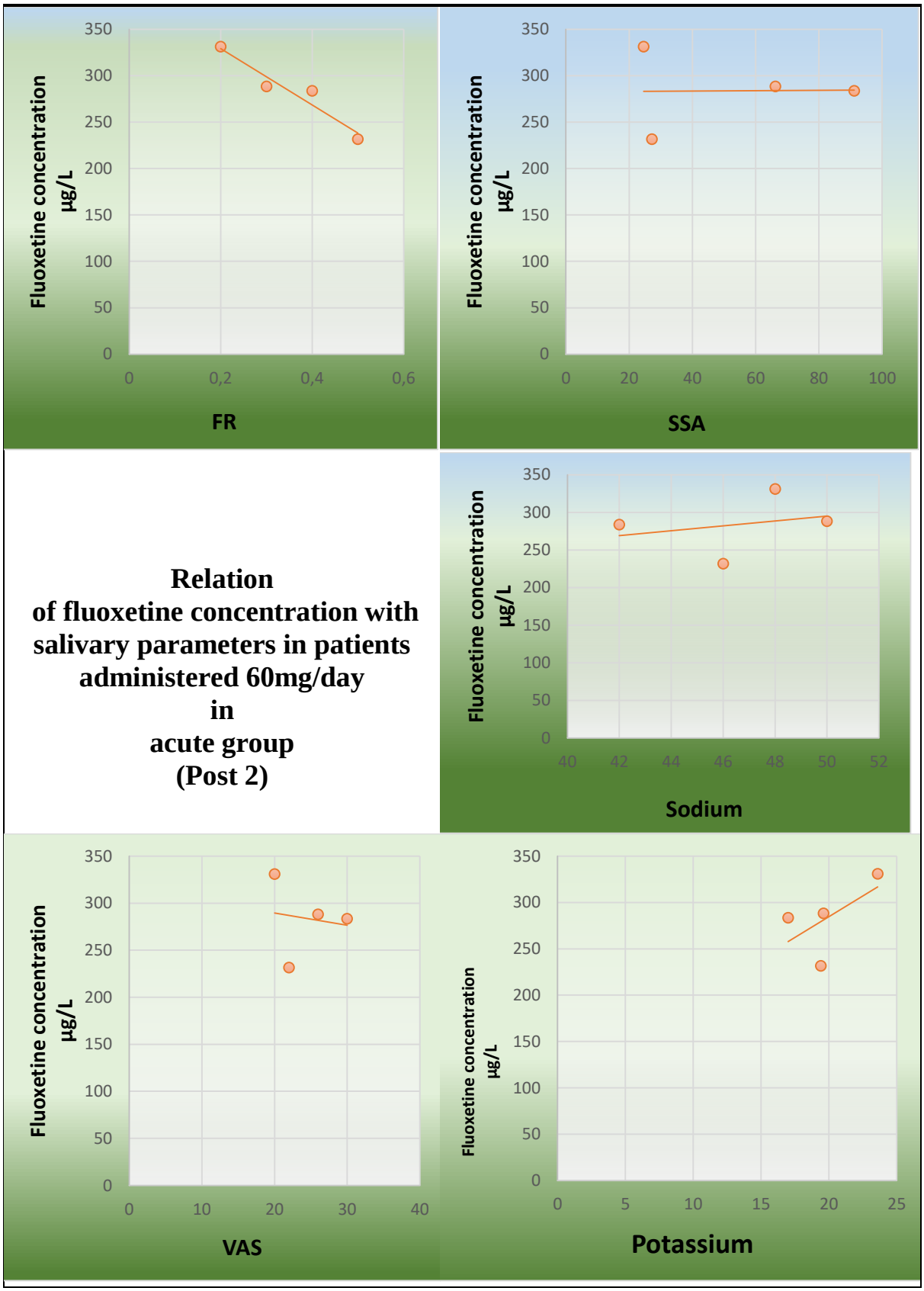

Figure 4.22: The relation between fluoxetine concentration and salivary parameters in theAcute Group at dose 60 mg/day, post2 after two months of therapy.

5. DISCUSSION

5. DISCUSSION

5.1 Patient characteristics

The psychiatric disorder such as depression, anxiety, panic disorder and OCD represented a widely distributed disorders throughout the world. They may interfere with the patient's life and even they may affect their quality of life. Psychiatric disorders may affect the individual mainly in two ways:

a) The disease itself may make the patient suffer from many symptoms which varied in their severity. Also the disease process may produce many biochemical changes in the patients.
b) The drugs used to treat such conditions, such as fluoxetine may cause adverse effects on the patients and may cause biochemical changes on their bodies.

Sixty patients were enrolled in this study; their average age were 34.95 years which had agreements with Alonso *et al.* (2004 which indicate the prevalence of psychiatric disorders in population. The distribution of patients in relation to the type of psychiatric disorder (74.9% suffered from depression; 16.6% anxiety disorders; and 3.3% of OCD and panic disorders) was in agreements with Commonwealth of Australia (1999) which reported that the prevalence of depression was higher than other psychiatric disorders. Also according to According to the World Health Organization (WHO, 2010), major depression also carries the heaviest burden of disability among mental and behavioral disorders and according to (WHO, 2017), depression fact sheets which consider depression as depression is a common mental disorder. Globally, more than 300 million people of all ages suffer from depression, and is the leading cause of disability worldwide, and is a major contributor to the overall global burden of disease.

5.2 SAA as a biomarker of stress

Self-reporting subjective questionnaire forms in stress evaluation and as psychological diagnostic mean provide highly inconsistent results according to patient’s mood and attitude. And this is because many individuals suffering from stress related problems have a tendency to either negate or exaggerate the real condition; which may lead to a deviation in the study and discompose the results. Thus there is a high need for studying the changes caused by stress in the human body to evaluate the usefulness of the

bio physiological indicators or biomarkers to provide a reliable objective evaluation and assessment of a stress and psychologically related conditions.

Salivary biomarkers are one of most widely researched area of interest because it might to provide a reliable, noninvasive and objective measurements of the response of the body. The release of salivary alpha amylase enzyme was reported to react to physiological and psychological stressors. Thus it might be a biomarker of stress which consider very important and valuable biological markers in psychophysiological research and clinical practice. Salivary alpha amylase activity of the patients included in the study showed statistically significant higher values when compared to control healthy group at ($p=0.0001$). Also when comparing the SAA activity of the patients with the healthy levels, we notice that SAA had the ability to distinguish 76.9% of the patients at pretreated level and 85.7% of the patients in the chronic group. These percentages were increased by using the SAA, Na+ and K+ concentration measurements together to detect psychological patients. For these reasons, they might be considered as a good biomarkers of stress in psychological conditions.

Depending on the physiological response to stress, we can explain the increment in the SAA activity. Psychosocial stress is known to induce various responses of physiologic systems with particular increasing activities in the hypothalamus-pituitary-adrenal axis (HPA) reflected by cortisol secretion (Koray, 2003) as well as in the sympathetic-adrenal-medullary (SAM) system reflected by salivary alpha amylase level (Chatterton *et. al*, 1996). Many studies had shown that salivary alpha amylase reflected the adrenergic activity and thus might be used as a reliable index of the SAM (sympatho-adrenal medullary system) activity during stress. (Nater *et al*., 2005; Ehrlert *et al*., 2006; Van Stegeren *et al.*, 2006).

Chatterton *et al.*, in his study in 1996 suggest that alpha amylase levels reflect the reaction of a different stress system than HPA axis. Ravindranath *et al.* (2014) found that salivary alpha amylase activity increases in patients with chronic psychosocial stress and may be used as a biomarker of chronic stress.

All these studies support our finding that SAA activity represent a good biomarker of stress and can be used in conjunction with the questionnaire form to detect psychological patients under stress. Our sample size was less to represent the general population, but it gives an idea about the salivary changes that accompanying the stress and psychological conditions.

Many studies study the salivary activity after acute stressor physical or psychological (Granger *et al.,* 2006; Nater *et al.,* 2006 Malamud and Rodriguez-Chavez, 2011). Granger *et al.* in the study done in 2007 said "An increase in SNS activity leads to higher levels of alpha-amylase production, which can be measured by examining saliva samples "In this way, multiple studies have examined the relationship between norepinephrine and SAA because both are associated with SNS activation. However, the results have been inconsistent (Chatterton *et al*., 1996; Nater *et al.,* 2006). Allwood *et al*., (2011) suggesting that SAA is more reactive to laboratory stressors (performance or peer rejection tasks) than cortisol. This is the first study that examine the SAA activity in acute and chronic state and in psychological patients with different psychological situations and compare it with other salivary electrolytes and with the adverse effects reported in the patients.

5.3 Evaluation of the changes in the disease condition

The comparison between the pretreated baseline level before starting fluoxetine administration will clearly explain the effect of the disease process on the patients and as follows:

5.3.1 The biochemical changes occurred in the saliva of the patients

There are numerous studies on saliva due to their physiological importance. Hundreds of components help to detect systemic diseases and also provide biomarkers of health and disease status. It is critical for preserving and maintaining the health of oral tissues. However, it receives little attention until its quantity diminishes or its quality becomes altered.

Salivary testing in clinical and research settings is rapidly proving to be a practical and reliable means of recognizing oral signs of systemic illness and exposure to risk factors. The components of saliva act as a "mirror of the body 's health", and the widespread use and growing acceptability of saliva as a diagnostic helps individual, researchers, health care professionals and community health programs to detect, to monitor diseases and to improve the general health of the public. Some of these components were used in this study to detect the changes occur in the saliva due to drug therapy (fluoxetine) (Höld *et al.,* 1999).

5.3.1.1 Changes in SAA, Na^+ and K^+

In this study, significant changes were found between the pretreated level in a psychiatric patients and the healthy control individuals at $p < 0.05$ in the concentrations and output of SAA which was higher in the patients than in the healthy subjects. This had agreement with many studies related to the increment in SAA to body response to stress (Rohleder *et al.,* 2004; Takai *et al.,* 2004; Nater *et al.,* 2005; Nater *et al*, 2006; Ehlert *et al*., 2006).

Changes in SAA are thought to have implications for health, for example, two studies by Granger *et al*. (2006; 2007) suggested a link between SAA and disease.

Release of SAA is regulated by autonomic innervations (Bagan-Sabastian, 2004) and usually higher level of SAA is produced by increased sympathetic activity (Granger *et al*., 2006; 2007).

Stress response is regulated by two primary neuroendocrine systems:

1. The hypothalamus pituitary–adrenocortical axis, in which cortisol consider a good biomarker to its activity, and sympathetic adreno-medullary system, in which SAA level considered as a good biomarker to its activity (Friedlander and Mahler, 2001).
2. The changes in SAA levels may be related to the activation of the beta-adrenergic system and reflects the psychological stress in depression and other psychiatric disorders. However, the results of Kivlighan and Granger (2006) and Inagaki *et al.* (2010) indicated a predominant role of the sympathetic nervous system in the secretion of SAA together with parasympathetic withdrawal, under psychosocial stress, which support the suggestion that psychological factors (like stress) effects SAA secretions and concentrations.

Equally, significant changes were found between the pretreated level and the level in the control group in the concentrations of Na^+ and K^+. The Na^+ concentration was significantly high, while K^+ concentration was significantly low in the disease condition than in the healthy subjects.

Since most of the patients in this study were depressed and even other psychiatric disorders might have been associated with depression, the explanation would depend on this to explain our finding. Depression known to be associated with increased level of aldosterone which lead to increase Na^+ reabsorption and exchange of K^+ this will explain the increased level of Na^+ and decreased level of K^+ in the disease condition when compare with healthy individuals (Zanatta *et al.,*2001).

5.3.2 Changes in the salivary flow rate and VAS

Multiple systemic disorders and medications have been reported to cause xerostomia and/or salivary gland hypofunction (Navazesh *et al*., 1996). Also, Rantonen (2003) considered that higher proportion of psychiatric illnesses in the hospitalized patients could be a factor simultaneously affecting salivary flow rate. The measurement of salivary flow is also important, because the concentration of various components of saliva like SAA is markedly affected by variation in flow rate (Kaite *et al*., 2008).

This may explain our finding that although there were no significant differences between the disease and the healthy conditions, but the FR in the pre level was less than the control healthy condition. This has an agreement with Hunter and Wilson (1995), who stated that depression itself contributed little to the oral dryness observed in depressed patients or reported by them. They also found that the patients subjective rating of oral dryness related well to a reduction in stimulated flow, this was in agreement of our finding that significant changes were reported in the disease condition which were higher VAS score than the healthy condition and this also agreed with many studies (Wewers and Lowe, 1990; Eisbruch *et al*., 2001; Jabbari *et al*., 2005; Meirovitz *et al.*, 2006) which found that VAS or self-reported questionnaire was a good and useful indicator of mouth dryness.

5.3.3 Evaluation of the clinical symptoms

Nervousness, constipation, palpitation, suicidal thoughts, headache, insomnia, change of appetite: all have been reported in the pre level group. Similarly, adverse consequence has been documented in the American Psychiatric Association which published the criteria for diagnosis of depression (Hirschfeld *et al.*, 1997) and in Adams (2003).

Black hairy tongue, xerostomia, dysgeusia had been reported in the pre level. Xerostomia have agreement with Hunter and Wilson (1995) who said that depression itself contribute little to the oral dryness reported by the depressed patients.

5.4 Evaluation of the changes in the treated groups with fluoxetine (Chronic and Acute groups)

It is suggested that (8 weeks) is sufficiently long enough to register clinically significant changes in the parameters studied.

5.4.1 The biochemical changes occurred in the saliva of the patients

Significant differences have been reported in the chronic group and higher level than the non-treated (control and pretreated level). Also, significant differences have been reported between different readings (pre, post1, post2) of the acute group in the SAA output**.**

Although the level showed decliner with the treatment, but it still higher than the healthy level. In this study, it was found that the SAA output was significantly higher in the disease condition (represented by acute and the chronic group in this study) compared with the healthy control condition. This finding was approved with Chatterton *et al.* (1996; 1997) which linked levels of SAA to sympathetic activation during physically and psychologically stressful conditions. The level of SAA was observed to increase in an investigation that used written examinations as a psychological stressor (Kelly *et al*., 2010).

Significant differences were found between the base line pretreated level (19.87u/min) which was higher than the treated post1 (12.86 u/min) and post2 (11.43 u/min) measures which indicated the efficacy of the treatment to reduce psychological stress associated with the psychological conditions. This is in agreement with (Noto et al., 2005) were they found that salivary alpha amylase is a useful indicator of psychological stress.

The SAA level was higher in men than women. This agreed with Duskova *et al.* (2010) which mentioned that the biochemical marker such as SAA differ between men and women.

As discussed above, the changes in SAA levels may be related to the activation of the beta-adrenergic system and reflects the psychological stress in depression and other psychiatric disorders. However, the results of Kivlighan and Granger (2006) and Inagaki *et al*. (2010) which indicated a predominant role of the sympathetic nervous system in the secretion of SAA together with parasympathetic withdrawal, under psychosocial stress, support our suggestion that psychological factors (like stress) affect SAA secretions and concentrations. Thus, as a result to body response to fluoxetine therapy and their efficacy in treating psychological disorders such as depression and improved in mood due to the increased 5-HT level and reduce the effect of stress on the patients. This will have appeared in the decliner in SAA secretion which reflect the decrease in sympathetic activity.

Fluoxetine act on the neurotransmitter serotonin which is normally released into the synapse between the nerve cells and is either destroyed or reabsorbed back into the cell that released it. Fluoxetine block this reuptake causing more serotonin to accumulate in

the synapse, increasing the level of serotonin in the synaptic cleft available to bind to the postsynaptic receptor thus the concentration of serotonin in the cleft is heightened and neuronal activity is enhanced.

On the other hand, in the chronic group, Na^+ and K^+ concentrations reported significant differences in related to the pre level group. The concentration of Na^+ was less than in the disease condition at the pre level but it still higher than the healthy level. While K^+ reported higher level than the pretreated levels, but still less than the healthy condition.

Also, Na^+ reported significant changes between pre and post treated level in the acute group while K^+ showed no significant changes. These findings have agreement with the study done by Hunter and Wilson (1995) who found that SSRIs treatment did not induce significant changes in Na^+, and K^+ concentrations and in salivary flow rate in patients using fluoxetine or paroxetine in a controlled clinical trials for two months duration.

Published studies do not offer any explanations for drastic changes in K^+ and Na^+ concentrations in the psychiatric patients on fluoxetine therapy. It is interesting that many of patients in this study showed salivary changes such as significant changes reported between the disease condition (patients chronic and acute groups which was on fluoxetine therapy) when compared with the healthy control subjects. Based on published studies on non-psychiatric patients, it is attempted to supply some explanations. As the salivary flow rate increases, the concentrations of total protein, Na^+, calcium, chloride and bicarbonate, as well as the pH increases to various levels (Edgar, 1992; Tenovuo *et al*., 1994) which may explain these findings that the Na^+ showed a pattern of decliner with the treatment and it reached to the healthy control level in the post 2 level. This may be as a result of the decreased in salivary flow that have been reported with treatment and this was also in agreement with (Höld *et al*., 1999) which propose direct relation of Na^+ secretion with the salivary flow rate. This also may explain the pattern of increment with treatment that have been reported with fluoxetine therapy which may be due to the indirect relation of K^+ secretion with FR as mentioned by Höld *et al*., (1999). Another explanation to the decrease and increase in Na^+ and K^+ concentration respectively could be related to the hormonal modulation of the saliva composition (Wotman *et al.,* 1973; Kakmoto *et al.,* 1988), since it acts directly increasing the Na^+ reabsorbtion and K^+ secretion. Also this supported by different studies (Zanatta *et al*., 2001) which found that fluoxetine increases the Na-K ATPase activity by 27% than the control which might lead to increase Na^+ reabsorption and K^+ excretion.

According to all of the above, and by comparison of the concentrations of Na^+ and K^+ with the concentrations mentioned in other studies we can notice the higher concentrations of these parameters in our sample compared with others, which may be a serious or even dangerous indicator to the severe and continuous stress that our population live in. This exciting finding may give rise to many studies that should be done to investigate the effect of such acute and continuous stress on the performance and well-being in our population and its effect on cognitive functions and memory. Also, on the ability to make a decision. According to this point of view, that needs more studies to measure the effect of acute and chronic stress and anxiety in our population, not only to study it, but to try to find some solutions or methods to compensate or deal it.

5.4.2 Changes in the salivary flow rate and VAS

Related to changes in the salivary flow rest and subjective perceptions (VAS) at rest, without exogenous or pharmacological stimulation, there is a small, but continuous salivary flow, denominated basal unstimulated secretion. This secretion, in the form of a film, covers, moisturizes, and lubricates the oral tissues. Both flow rate and VAS results showed significant differences between the three groups in this study. Higher salivary flow rate was recorded in the healthy subjects and lower in the psychiatric patients treated with fluoxetine. On the contrary the VAS scores in the healthy control subject were lower compared to those obtained for the group of patients with psychiatric disorders.

Significant changes had been found between the chronic groups and the pretreated level and the healthy control level. According to previous studies (Friedlander and Mahler, 2001), significant differences have been reported between pre and post treatment level with fluoxetine as). The results obtained were also agreement with Bretz *et al*. (1993) which found that FR showed significant changes after fluoxetine therapy and with the study of Hunter and Wilson (1995) who found that fluoxetine therapy caused reduction in the salivary flow of the parotid gland but this reduction was not significant.

VAS was used in this study as a subjective measure of the feeling of the patients of xerostomia had a good correlation with the salivary flow and with the reduction in salivary flow. Salivary flow reduction interferes with the functions of saliva in the patients lead to disturbance in food digestion and mastication and may even interfere with the patients sleep leading to sleep disturbances.

Significant differences were reported between the healthy and disease condition and also between pretreated level and post fluoxetine treatment levels.

The unstimulated saliva flow rate in this study was somewhat higher than reference values for unstimulated flow, 0.25 – 0.35 ml/min (Ericson and Mäkinen, 1986). It is obviously practically impossible to obtain true unstimulated saliva, since saliva flow is always influenced by some kind of stimulation. Even slight movements of the tongue, cheeks, jaws or lips should be avoided (Tenovuo and Lagerlöf, 1994).

The range of normality is so broad that no reference values exist for the whole population (Ship *et al.*, 1991). In this study, female showed lower salivary flow rate than male in all groups; as in many studies which showed that females had lower salivary flow rates than males (Percival *et al*., 1994; Tarkkila *et al*., 2001). These differences have been attributed to two theories; women present smaller salivary glands in comparison with men (Dawes *et al.*, 1978), and the female hormonal pattern may contribute to diminish salivary secretion. Tarkkila *et al*. (2001) and Percival *et al*. (1994) found that healthy, non-medicated women presented a lower mean for total unstimulated SF and for stimulated SF of the parotid when compared with men. Whereas, Shern *et al*. (1993) reported the total unstimulated salivary flow was not influenced by gender.

As mentioned before, saliva plays important role in regulating oral health and the physician does not have an attention unless its quantity in the mouth decline. Oral health is an integral and critical part of general health. Depression and other psychiatric disorders can affect oral health and lead to xerostomia, alterations in salivary composition and flow rate, and adverse effects related to drug therapy include oral infections, increased susceptibility of dental caries and bruxism. Although oral health problems rarely are serious, they may have significant social, economic and psychological consequences for patients, and may affect their quality of life.

As patient management shifts from treatment to preventive models, the detection, recognition and prevention of salivary gland hypo function in depressed and psychiatric patient which is more susceptible to it as a result of both the disease condition and the drug therapy, will become more important.

The selective serotonin reuptake inhibitors SSRIs (fluoxetine) exert their antidepressant effect by preventing presynaptic neurons from reabsorbing (reuptake) serotonin from the synaptic cleft for recycling. Thus, the concentration of serotonin is increased (Depattista, 2007).

The increased incidence of breathing difficulties, abnormal dreams, drowsiness, insomnia, headache, weight loss, change in appetite, tremor, myalgia, anorexia, nausea, diarrhea, and anxiety that reported in the chronic group and in the post 2 (after 2 months

of treatment of fluoxetine) represented mainly the adverse effects caused by fluoxetine therapy. These adverse effects were mild to moderate in severity. Our observations are in agreement with Birmaeher *et al*. (2003) who studied the side effects of fluoxetine versus placebo; with many clinical trials introduced by the Elly Lilly company to get FDA approval in 1987; placebo-controlled clinical trials (March *et al*., 2004; Safer, 2006); and also with many researches (Goldstein and Goodnick, 1998; FDA patient information sheet, 2006).

The direction of causality cannot be determined, since, it is difficult to tease apart the effects of fluoxetine from the effects of the disease process itself, such as, suicidal ideation which has been frequently reported as a side effect of fluoxetine. However, this is also a common symptom of depression itself (American Psychiatric Association, 2000) and (Cohen, 2007) which reported increased suicidality in adolescent consuming fluoxetine.

5.4.3 Evaluation of the clinical adverse effects reported with fluoxetine therapy

Related to withdrawal from fluoxetine therapy (41.02%) of the selected 39 patients to be followed up for two months duration was withdrawn from our study for different causes, Only 23 patients complete the two months acute duration. This is considered a high withdrawal percentage in comparison with other studies. As it appears in the results section (pag. 85-87).

It is relatively agreed with Birmaher *et al*., (2003) who studied the adverse effects of fluoxetine versus placebo and it agreed also with the Expert Panel Monograph in 2004 which reported that insomnia, GIT, and sexual adverse effects might be the main causes of fluoxetine withdrawal. Moreover, Kauffman (2009) in a 6-week study reported a total dropout rates between 30% and 70% which some 30-40% are attributed to side effects and the rest to failure of treatments.

The assessment of human sexual dysfunction associated with fluoxetine therapy is complicated, because sexual dysfunction is common problem in the general population and can be associated with depression. However, effects of fluoxetine therapy on sexual function, i.e., ability to achieve orgasm, have been observed at doses of 20 mg /day or higher also data from experimental animals support the observations in human (NTP-CERHR, 2004). The finding of the current study agreed with the general finding in many studies such as (Grimsley and Jann, 1992; Wong *et al*., 1995; Stockes and Holtz, 1997; CERHR, 2004). Suppression of appetite and weight loss was reviewed by Goldestein and

Goodnick (1998). Also, this study might provide support for the 5-HT hypothesis of anxiety, which propose that increased level of 5-HT associated with anxiogenic effects, while reduction in 5-HT are associated with anxiolytic effects (Drapier *et al*., 2007).

In relation to the oral adverse effects of fluoxetine, the official U.S Food and Drug Administration medication package inserted the orofacial adverse effects accompanying each of the antidepressant medication that may occur (Pysicians Desk Refrence, 2000).

In this study, there is an increased incidence of glossitis, taste changes (dysgeusia), xerostomia, and TMJ problems and this have an agreement with many studies, such as (Ellingrod and Perry, (1994); Davindran *et al*. 1997a; Trinddade *et al*., 1998; Bagan-Sabastian,2004). Oral infection has showed to be significantly decreased with the treatment and it was higher in the pretreated level than in post fluoxetine treatment level, this finding was in agreement with Núñez (2010) who found that fluoxetine inhibit the *C. albicans* growth. Friedlander and Mahler (2001) reported similar orofacial adverse effects with fluoxetine therapy. Bostwick and Jaffee (1999) supposed that the cause of bruxism was due to the increase extrapyramidal levels of serotonin, there by inhibiting dopaminergic pathways that control movements.

5.5 The Effect of gender in fluoxetine therapy

Sex differences in response to fluoxetine have been previously documented by Laroche and Morgan (2007). In our study we found that depression is more prevalent in female than in male (WHO, 2008), while anxiety and OCD are more prevalent in male. Many theories have been developed to explain such differences based on biological, environmental and psychological causes. Nolen-Hoeksema, (1990) and Blazer *et al.*, (1994) have shown that women are roughly twice as likely as men in experience of depression. While only Shalini *et al.* (2011) reported that men suffer from depression was higher than women. Also a relationship between 5-HT and estrogen and mood regulation has been previously being described in both human and animal studies (Rubinow and Roca, 1998). Suggesting that determinants of gender differences in common mental disorders are still far from being understood (Klose and Jacobi, 2004).

5.5.1 Evaluation of the biochemical changes in saliva

The pattern of changes in the salivary parameters were the same in the healthy subjects and the patients in our study. Salivary amylase, Na^+, and FR were higher in males than in females, while potassium and VAS were higher in females than in males. Percival

et al. (1994) and Pavinum and Larmus (1981) also, found that healthy non-medicated women presented a lower mean for total unstimulated salivary flow rate and for stimulated salivary flow of the parotid when compared with men. Dawes (1996) and Jehi-Petri *et al*. (1997) were also in agreement with our findings that salivary flow rate was higher in males than in females. In the other hand, our finding that SAA was higher in male than in female is consistent with the study of Vigil *et al.,* (2010).

5.5.2 Evaluation of safety and tolerability of fluoxetine between male and female

Nausea, change appetite, myalgia showed higher incidence in female than in male. While male showed higher incidence of anxiety and nervousness so as taste changes (dysgeusia). Sex differences in response to fluoxetine have been previously documented by Laroche and Morgan (2007).

5.6 Evaluation of the effect of different doses on salivary flow and content and its relation with the adverse effect of fluoxetine

Generally, no significant differences in salivary parameters in our patients reported in relation to dose, only Na^+ concentrations showed an increase level in dose 40 mg/day.

Most of the adverse effects of fluoxetine showed slight increase in incidence associated with the increased in dose of fluoxetine. Only anxiety showed significant statistically higher incidence with the dose 60 mg/day from the general and oral adverse effects that have been reported in our study. Fluoxetine adverse effect like other SSRIs is mostly dose dependent, appear in up to 75% of patients on normal doses (Kauffman, 2009).

5.7 Evaluation of the effect of different duration of fluoxetine therapy on salivary flow and content and its relation with the adverse effect of fluoxetine

SAA and Na^+ and k^+ concentrations showed no significant differences between groups in relation to different duration of fluoxetine therapy.

The FR decreased with the duration and the lowest FR reported was 0.16 ml, while the VAS showed high scores with long term treatment which was (40.50).

Weight loss, myalgia and nausea significantly increased with the treatment duration (Ferguson, 2001). Fluoxetine is more likely to produce appetite suppression and weight loss is reviewed by Goldstein and Goodnick (1998) leading to off label use of this medication in obesity.

While breathing difficulties, nervousness, insomnia and anxiety appeared to be high in the pretreated base line period as an effect of the disease, as a symptom (Moret and Isaac, 2007). They showed decline in the short term therapy of fluoxetine and then show an increment with the long duration. This has an agreement with (Buchman *et al*., 2002) which suggest the existence of a late-onset side-effect profile, which appears similar to acute side-effect symptomatology. Super sensitivity of the serotonin-related receptors may develop over the long-term and account for the phenomenon. And they report for the first time on two cases of late-onset adverse effects occurring 6 and 10 years after chronic-fluoxetine treatment in which patients experienced symptoms of restlessness, tension, agitation, and sleep disturbances.

Taste changes, oral infection, xerostomia, TMJ problems showed significant increased with the increased duration of therapy. This may occur, because this medication increase extra pyramidal levels of serotonin, thereby inhibiting dopaminergic pathways that control movements (Friedlander, 2001; Guggenhiemer, 2003).

5.8 Implication of oral side effects of SSRIs and their managements

Depression is regularly treated with antidepressant medication, which has been shown to have numerous side effects, some of which affect dental health. Xerostomia puts the individuals at greater risk for tooth decay, periodontal diseases, and increased requirements for periodontal treatment, dental restorations, and dental execrations (Kenkre and Spadigam, 2000; Friedlander and Marder, 2002).

Major depressive disorders (MDD) may be associated with extensive dental disease, and people may seek dental treatment before becoming aware of their psychiatric illness. MDD frequently is associated with a disinterest in performing appropriate oral hygiene techniques and to follow a cariogenic diet, which diminished salivary flow, induces rampant dental caries and advance of periodontal diseases.

A diminished volition in individuals with severe mental illness may affect their ability and desire to perform preventive oral hygiene procedures (Friedlander and Liberman, 1991; King, 1998; Friedlander and Mohler, 2001). Furthermore, increased use of candy, chewing gum, and carbonated beverages to combat xerostomia can further promote tooth decay (kenkre and Spadigam, 2000). Since many oral and systemic conditions manifest themselves as changes in the flow and composition of saliva, the psychiatrist is advised to remain up-to-date with the effects of diseases and their treatments on saliva and on the oral health of the patients.

We must emphasize that dentistry, in concert with medicine, have much to offer to patients with psychiatric illness. Our goal is to encourage psychiatrists to recognize patients with occult oral health problems, make knowledgeable referrals to dental health practitioners for confirmation of the diagnosis and treatment, and to offer these patients the full range of dental treatment options.

5.9 The relations of fluoxetine concentrations measured by HPLC with dose, gender, SAA, salivary electrolytes and with the adverse effects

All patients being treated with antidepressants for any indication should be monitored appropriately and observed closely for clinical worsening, suicidality, and unusual changes in behavior, especially during the initial few months of a course of drug therapy, or at times of dose changes, either increases or decreases. For that purpose, HPLC is the most commonly used mean for quantitative serum measurements of a medications.

Measurement of the drug is useful for managing co-medications, dose or formulation changes, and in assessing compliance. Therapy with fluoxetine is subject to numerous drug interactions, which is compounded by wide inter individual variability in fluoxetine pharmacokinetics such as a half-life and metabolism. Fluoxetine is a potent inhibitor of the metabolic enzyme CYP2D6, with lesser inhibitory effects on CYP2C19 and CYP3A and due to the long half-lives of parent and metabolite (1-6 days), it may take several weeks for patients to reach steady-state concentrations

Previous studies have examined the relationship between fluoxetine concentrations and therapeutic response. Some studies like (Altamura *et al.,* 1994; Fichtner *et al.,* 1992; Cain, 1992) have suggested a curvilinear relationship between clinical response and plasma concentrations, while Norman *et al.,* 1993 in his study "Relationship between antidepressant response and plasma concentrations of fluoxetine and norfluoxetine" said no relationship between fluoxetine concentrations and response. The same things found previously by Kelly *et al.,* 1989. Thus determination of the relationship between fluoxetine concentrations and clinical response remains a questionable and also the relations between fluoxetine concentration and the adverse effects reported by patients.

Many factors might influence the measured plasma drug concentrations, including variations in absorption, distribution, and metabolic clearance rates that are influenced by genetic and other differences among individuals.

In this study, a wide range of fluoxetine concentration have been reported in relation to dose. All was within the reported therapeutic levels (Montgomery *et al*., 1986).

In general, the mean concentration reported in this study was higher in females than males in the chronic and acute groups. Many studies mentioned that sex differences were observed in relation to dose and fluoxetine serum concentration. (Blazquez *et al*., 2014 and Ferguson, 2006). While, Pato *et al.,* 1991 in his study said that sex and age of the patient did not impact metabolism and drug concentrations of fluoxetine

Amsterdam *et al.,* 1997 also support our finding and had found in his study that males had lower fluoxetine and norfluoxetine serum levels than females.

In our study, no clear relation noticed between drug concentration and a patients' withdrawal from the study but these patients reported a higher range of fluoxetine concentration in the previous reading comparing with other patients. Amsterdam *et al.* (1997) reported that decreased clinical response at higher plasma concentrations and Altamura *et al.* (1994) was find that concentrations of fluoxetine plus norfluoxetine above 500 mg/L appear to be associated with a poorer clinical response than lower concentrations. Montgomery *et al*. (1986) were the first to provide evidence of a therapeutic window for fluoxetine. They studied the plasma concentration response relationship in two groups of patients. The first group was treated with fluoxetine 60mg/daily, and the second group of patients received fluoxetine 80mg once weekly. They found that mean plasma concentrations ranged from 200 to 531 mg/L for fluoxetine and from 103 to 465 mg/L for norfluoxetine after the first week of therapy. No relationship was seen between plasma fluoxetine concentration and response, but a significant negative relationship was observed between plasma concentrations of norfluoxetine and response. The group of patients that responded at the end of the study had significantly lower plasma norfluoxetine concentrations than non-responders.

Also no significant relation has been found between fluoxetine concentration and general adverse effects in this study and this is in agreement with Beasley (1990) which discuses "fluoxetine: relationships among dose, response, adverse events, and plasma concentrations in the treatment of depression" and found adverse events were not related to plasma concentrations. While Altamura *et al*. (1988) reported the increase in incidence of nausea and vomiting with the increase in fluoxetine dose and said that "It is unclear whether common adverse effects of fluoxetine, including nausea, are related to plasma concentrations of the drug. However, it is clear that with higher dosages of the drug, the incidence of nausea and vomiting increases". Furthermore, according to Fichtner *et al*. study in 1991, which hypothesized that suicidal ideation may occur with high plasma fluoxetine concentrations

Direct relation was found between plasma concentration of fluoxetine and oral adverse effects (xerostomia); the incidence of xerostomia was increased at the higher drug concentrations in our study. Also salivary flow rate showed decrement with the higher fluoxetine concentrations. This symptom may be the result of both diminished salivary secretion and an alteration in saliva composition (Patrícia *et al.,* 2012). The subjective dry mouth sensation may occur even in the presence of a normal salivary flow that is, not necessarily being associated with a diminution in the amount of saliva (Närhi, 1994).

The antidepressants inhibit the cholinergic signals in the salivary tissues and thus diminish the excretion of fluids by the glands, and interferences in central pathways (serotoninergics and dopaminergics) may also alter salivary composition (Atkinson and Baum, 2001).

It is important to emphasize that the dry mouth sensation and alteration in salivary composition may occur during periods of stress and/or acute anxiety, frequently present in depressive disorders, due to predominant stimulation of the sympathetic system, irrespective of the use of anxiolytic and/or antidepressant medication (Guggenheimer and Moore, 2003). Therefore, it may be difficult to determine whether these side effects and their intensity arise from the medical condition that led to the treatment, or from the medication prescribed for it (Smith and Burtner, 1994), it probably is as a result of both (Patrícia *et al.*, 2012).

6. CONCLUSIONS

6. CONCLUSIONS

Teniendo en cuenta los resultados de este estudio y la discusión de los mismos, se pueden obtener las siguientes conclusiones:

1. La evaluación de la cantidad y análisis de la composición de la saliva podrían ser considerados como un método analítico, no invasivo, eficaz para el estudio de los cambios originados en el estado de los pacientes psiquiátricos, así como en la evolución de su tratamiento.
2. Se han encontrado diferencias significativas en la concentración salivar de SAA, Na^+ y K^+ entre los voluntarios sanos, los pacientes con alteraciones psicológicas, y los pacientes tratados farmacológicamente.
3. La medida de la concentración de alfa amilasa salivar (SAA) puede considerarse un adecuado biomarcador para evaluar el estrés en pacientes con alteraciones psicológicas. También puede ser empleado para evaluar la eficacia de los tratamientos farmacológicos sobre el grado de estrés de los pacientes.
4. A pesar de que el tratamiento con fluoxetina origina una disminución significativa en la liberación de SAA y en la concentración de Na^+, no es posible establecer una clara correlación entre su concentración plasmática, determinada en los pacientes tratados, con los biomarcadores de estrés y/o con la incidencia de efectos adversos. Por ello, no es posible su consideración como estimador para el seguimiento de la evolución del estado psicológico de los pacientes, ni para la toma de decisión en el tratamiento.
5. Se ha observado una correlación directa entre la concentración de Na^+ y la medida del flujo salivar.
6. Los resultados obtenidos, tras la aplicación a los pacientes de la escala analógica visual (VAS), ha mostrado una buena correlación con los cambios observados en el flujo salivar. En pacientes enfermos, valores elevados en VAS están relacionados con una disminución en el flujo de saliva. Tras el tratamiento con fluoxetina se observan cambios significativos en los valores de VAS y en el flujo salivar. Por ello, esta escala puede ser considerada una buena herramienta para la estimación subjetiva de la percepción de sequedad de boca.
7. En este estudio el porcentaje de abandono del tratamiento farmacológico se ha estimado en un 41.02%, de los cuales un 20.5% ha sido consecuencia de los los efectos adversos provocados por el tratamiento, especialmente trastornos

gastrointestinales, insomnio y alteraciones en la función sexual. El abandono del estudio como consecuencia de alteraciones en la articulación temporo-mandibular (TMJ) ha sido del 2.56%. Xerostomia y disgeusia son efectos adversos asociados al tratamiento con fluoxetina.

8. El género influye significativamente tanto en el abandono del tratamiento como en la incidencia o percepción subjetiva de efectos adversos. Se observó un mayor número de abandonos en los varones. Sin embargo, en el grupo de mujeres, los valores de VAS fueron más elevados, por lo que parecen ser más sensibles a la percepción de sequedad de boca.
9. Se constata la escasa información científica publicada acerca de los efectos adversos originados en la cavidad oral por los tratamientos con antidepresivos y en especial por inhibidores en el re captación de serotonina (SSRIs), así como sobre su posible prevención y tratamiento. Para mejorar ese conocimiento se proponen las siguientes recomendaciones:

    - En los registros del seguimiento diario de los pacientes con alteraciones psiquiátricas debería incluirse información sobre la higiene de la cavidad oral y los efectos de la medicación sobre la misma.
    - Sería conveniente alentar a los pacientes para que se preocupen por la higiene y salud de su cavidad bucal como parte importante de su estado saludable o como manifestación de algún problema psiquiátrico. Fomentar en ellos la importancia de comunicar cualquier incidencia al médico.
    - Crear programas educativos dirigidos a los ciudadanos para controlar el estrés y para manejar convenientemente las consecuencias psíquicas y físicas del mismo.
    - Implementar programas de formación continuada para profesionales sanitarios que les permita la actualización de conocimientos sobre los síntomas orales, tanto de los trastornos psiquiátricos como de los efectos adversos inducidos por los tratamientos.

Consideración final:

Este estudio, a pesar ser un estudio preliminar, limitado por el tamaño de muestra, puede contribuir, por su diseño y resultados obtenidos, al hallazgo de nuevos biomarcadores que permitan establecer los niveles de estrés de la población mediante análisis sencillos y de alta capacidad, como es la evaluación de la composición de la saliva. No obstante, se hace necesaria la realización de una investigación más amplia mediante la cual, no solo afiancen los resultados obtenidos, sino que permita el estudio de los efectos adversos originados sobre la cavidad oral por los tratamientos farmacológicos de enfermedades como la depresión y la ansiedad, facilitando con ello su prevención y tratamiento.

Se apunta la posibilidad de evaluar en el futuro la incidencia de la violencia y en concreto los efectos de la guerra en el mismo grupo de población, individuos residentes en la ciudad de Mosul (Irak), con objeto de establecer posibles diferencias relacionadas con estados de estrés crónico de grado máximo.

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