

Depressive symptoms, monoamines levels, MAO-B activity and effect of treatment in a subset of depressed individuals from government sector hospital at Karachi

Farah Zeb¹, Sabira Naqvi¹, Raza ur Rahman² and Ahsana Dar Farooq^{1,3*}

¹H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

²Dow University of Health Sciences, Karachi, Pakistan

³Hamdard Al-Majeed College of Eastern Medicine, Hamdard University, Karachi, Pakistan

Abstract: Depression is one of the leading causes of disability in developing countries including Pakistan. This study was designed to assess the frequency and severity of depressive symptoms, monoamines and their metabolite levels, MAO-B activities before and after treatment with antidepressants in a sub-set of Karachi population in Pakistan. Drug naive depressed subjects were evaluated before and after treatment with selective serotonin reuptake inhibitors. Symptoms of depressed mood and anxiety psychic (90%) were more frequent whereas, suicidal thoughts (~50%) and feelings of guilt (~30%) were less common. Hamilton Depression Rating Scale scores were 21.4 ± 0.8 in both genders with a significantly higher score (1.3x) in females. Homovanillic acid, 5- hydroxyindoleacetic acid and MAO-B activity were significantly higher 43%, 66% and 25% respectively, in depressed than normal subjects. A significant decline after 2 weeks treatment in HDRS scores with fluoxetine (19%) and paroxetine (40%) and in MAO-B activity (20%) was observed. In conclusion, in our population early decline in HDRS scores supports that they are SSRIs responders, whereas a concomitant reduction in MAO-B activities indicates that it can be considered as one of the parameters for early detection of response. Additionally, the low frequency of suicidal thoughts could be associated with higher levels of monoamine metabolites.

Keywords: Depressive symptoms, HDRS-18, Karachi population, platelet MAO-B activity, plasma HVA, plasma 5-HIAA

INTRODUCTION

Depression, a mental health disorder affecting more than 350 million people around the world (WHO, 2012) is expected to rise from 4th to 2nd position among the leading causes of disability by 2020 (Murray and Lopez, 1996; Schutter and Honk, 2005). Life time prevalence of depression varies among different populations, which is partly based on environmental and/or genetic factors. About 50.8 million sufferers of major depression belong to developing countries (Gadit and Mugford, 2007). In different rural and urban communities of Pakistan, its prevalence is variable and has been reported as 6% (Gadit and Khalid, 2002), 34% (Mirza and Jenkins, 2004) and in gender based studies the values were 66% and 72% for females as compared to 25% and 44% males respectively (Mumford *et al.*, 1997; Niaz *et al.*, 2004). The pattern of double ratio between females and males has been reported persistently (Naqvi, 2007; Altaf *et al.*, 2015). In Karachi, a cosmopolitan city of Pakistan, with a population of over 20 million, the prevalence of depression was ~30% in an urban squatter settlement (Ali and Amanullah, 2000) and similar rates were observed in women from lower- middle class semi urban area as well (Ali *et al.*, 2002).

The Hamilton depression rating scale (HDRS) is considered to be most acceptable and reliable tool since 50 years (Hamilton, 1960). It allows the quantitative assessment of depressive symptoms including frequency (Jang *et al.*, 2011) and its severity (Figueras *et al.*, 1999). The variations in the depressive symptoms among different populations (Nakane *et al.*, 1991; Hang *et al.*, 2011) are influenced by many factors including cultures and traditions (Kastrup, 2011).

Apparently, biological hypothesis of depression involves monoamines including dopamine (DA), serotonin (5-HT) and their metabolites. In the serotonergic system mainly 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) are involved in behavior, appetite and sleep pattern (Barnes and Sharp, 1999). Their low levels have been associated with suicidal thoughts and severe depression (Brown and Linnoila, 1990). Furthermore, raised plasma levels of 5-HIAA in depressed patient reflect clinical improvement and response to the treatment (Saldanha *et al.*, 2009).

Monoamine oxidase (MAO), a flavin containing mitochondrial enzyme exists in two isoforms, MAO-A and -B characterized by their sensitivity towards specific substrates and inhibitors. MAO-A and -B preferentially deaminates 5-HT and phenylethylamine (PEA) and

*Corresponding author: e-mail: ahsanadar@hotmail.com

selectively inhibited at nanomolar concentration by clorgyline and selegiline, respectively. MAO has emerged as a very important biochemical tool in linking psychopathology, personality traits, and their biochemical bases (Grigorenko, 2002; Oreland, 2003). In the context MAO-B activity has been investigated widely using platelets as an easily accessible source, for analysis of various aspects of human mood and personality traits mainly including depression (Ziegelstein *et al.*, 2009 review), involvement in suicidal behavior (Verkes *et al.*, 1998) and anti social activities (Longato-Stadler *et al.*, 2002).

The antidepressant drugs remain the mainstay for the treatment of depression worldwide since the discovery of imipramine (tricyclic antidepressant, TCA) in 1950s. Selective serotonin reuptake inhibitors (SSRIs) introduced in 1980s and still used as first line medications because of its efficacy and tolerability (Anderson, 2000). Among SSRI group, although fluoxetine and paroxetine demonstrated similar effects against different depressive symptoms shows earlier improvement in the symptoms of agitation and anxiety psychic with later (Chouinard *et al.*, 1999).

There is no published information regarding depressive symptoms and its association with platelet MAO-B activity or with plasma levels of monoamines and their metabolites in Karachi population. Therefore these parameters were investigated in depressed and compared with normal subjects. Furthermore, the effects of antidepressant drugson these aforementioned parameters were also evaluated during a period of six weeks treatment.

MATERIALS AND METHODS

The drug naïve depressed individuals during 2005 to 2007 diagnosed by a psychiatrist from in- and out-patients of psychiatry department of Civil Hospital, Karachi, Pakistan were included in the study. The confidentiality and ethics were followed in accordance to the guidelines of the Ethical Review Committee, Dow University of Health Sciences, Karachi.

Inclusion and exclusion criteria

In this study, individuals after the diagnosis of depression according to ICD-10 criteria (WHO, 1993) and not receiving antidepressant drugs were included independent of parameters such as age sex and ethnic group. Furthermore, individuals having neurological or psychiatric disorder and medical illness, on any other medication or with a background of drug/substance abuse were not included in this study. Pregnant or lactating women or those using contraceptive pills were also excluded.

Selection of normal and drug naïve depressed individuals

The normal healthy volunteers (n = 52) including males (33) and females (19) of 31.4±2.1 and 29.7±1.7 years, respectively (range 18-70 years) were selected randomly. Drug naïve depressed (n=63) included in the study were consisted of males (n=45) and females (n=18), aged 48.1±1.7 and 39.0±3.4 years, respectively.

Consent forms and proformas

Prior to sampling, consent from normal and depressed individuals were taken on especially designed forms (local language Urdu / English) after explaining them the right of volunteers, purpose of the study and procedure of sample collection. Proformas were designed to collect their personal information (including name, age, sex, address etc.) along with present, past, family history and any drug history from diseased subjects.

Psychological assessment

All the individuals were assessed for the frequency of depressive symptoms before treatment, using HDRS-18 (Jang *et al.*, 2011). The total HDRS scores was employed for the evaluation of severity of these symptoms. For clinical improvement and responsiveness towards antidepressant drugs, these individuals were also assessed after treatment for a period of 2, 4 and 6 weeks. The treatment response was defined as a reduction in baseline scores $\geq 20\%$.

Chemicals

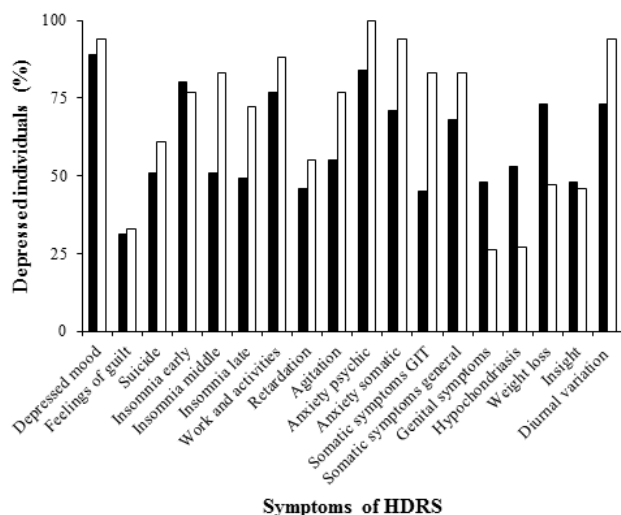
The chemicals used in the study were: 1-octane sulfonic acid, acetonitrile and methanol of HPLC grade (Fisher chemicals, UK), hydrogen peroxide solution (Merck, Germany), sodium azide (Ogawa Seiki Co. Ltd., Japan), hydrochloric acid (Ridel-de-Haën, Germany), 3, 4-dihydroxyphenylacetic acid (DOPAC) and dopamine hydrochloride (DA) (Sigma-Aldrich, Germany), 5-hydroxyindole-3-acetic acid (5-HIAA), 5-hydroxytryptamine (5-HT), adrenaline bitartrate, ammonium chloride, ascorbic acid sodium salt, benzylamine hydrochloride, ethylenediaminetetraacetic acid disodium salt, homovanillic acid (HVA), horseradish per oxidase (HRP), potassium bicarbonate, potassium chloride, potassium phosphate monobasic anhydrous, sodium chloride, sodium hydroxide, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic and α -D glucose (Sigma Co, St. Louis, Mo, USA). Deionized water (Millipore, simplicity 185 deionizer) to prepare solutions throughout the study.

Antidepressant drugs

The depressed individuals were treated with therapeutic oral doses of selective serotonin re-uptake inhibitors: citalopram, fluoxetine, paroxetine (20mg/day), sertraline (100mg/day).

Separation of platelet poor plasma and platelets

Platelet poor plasma was prepared as described earlier (Minegishi and Ishizaki 1984) with few modifications and used to determine levels of monoamines and their metabolites, while the platelets were collected to measure MAO-B activities. Briefly, blood sample (10ml) in EDTA.2Na (3%) was centrifuged (1500rpm for 10min) and plasma collected was further centrifuged (4000 rpm for 15 min). The platelet poor plasma (PPP) obtained was aliquoted (1 ml) and stored at -80°C till further analysis. Platelet-rich plasma (PRP) at the bottom was centrifuged (4500 rpm for 15 min). The supernatant obtained was again centrifuged (4500 rpm for 7 min) after addition of 2 ml phosphate buffer saline (PBS pH 7.3: KH_2PO_4 2.9 mM, $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ 25mM and NaCl 136mM). The pellet obtained was re-centrifuged (4500 rpm for 10 min) with lysis medium (NH_4Cl 155mM, KHCO_3 9.9mM and EDTA.2Na 0.09mM, pH 7.3) and finally centrifuged again with PBS (4500 rpm for 10min). Thus the pellet obtained was suspended in incubation buffer 500 μl (NaCl 137mM, $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ 12mM, KCl 2.6mM, KH_2PO_4 1.99mM and $\alpha\text{-D}$ glucose 5.5mM, pH 7.3) and visualized microscopically for identification followed by platelet counts to determine the conc /ml. The platelet preparation was aliquoted (200 μl) and stored at -80°C for measurement of MAO-B activity and used within a period of one week.



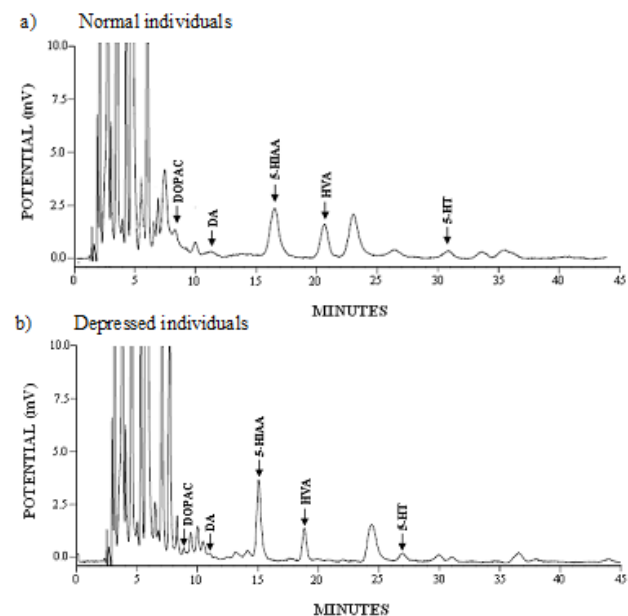
Depressed males (■, n = 45) and depressed females (□, n = 18)
Hamilton Depression Rating Scale = HDRS
Ranking of depressive symptoms = 1,2,3 according to frequency in descending order on the basis of cumulative percentage of both genders

* $p < 0.05$ and ** $p < 0.01$ represent significant differences in frequency of depressive symptoms between males and females

Fig. 1: Frequency of Hamilton Depression Rating Scale symptoms in depressed males and females.

Assay for monoamines and their metabolites levels in plasma

Platelet poor plasma (1ml) was mixed with 250 μl of hydrochloric acid (1M) and passed through the equilibrated solid phase extraction columns (Chromabond C18, 45 μm , 1ml/100mg, Macherey-Nagel, Germany) followed by washing with water (1ml). Finally the required metabolites were eluted with methanol (200 μl), filtered through syringe filter (0.22- μm) and a sample of 10 μl was injected into HPLC column.



Chromatograms obtained through high performance liquid chromatography with electrochemical detector (HPLC-ECD) to measure the levels of monoamines: dopamine (DA), 5-hydroxytryptamine (5-HT) and their metabolites: dihydroxyphenylacetic acid (DOPAC), 5-homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) in platelet poor plasma from normal and depressed individuals.

Phosphate buffer with acetonitrile (10 %, pH 3.4) was used at flow rate of 1 ml/min through reversed phase C_{18} column (250 x 4.6 mm). Electrochemical detector was set at 0.75 millivolts potential. Injection volume = 10 μl .

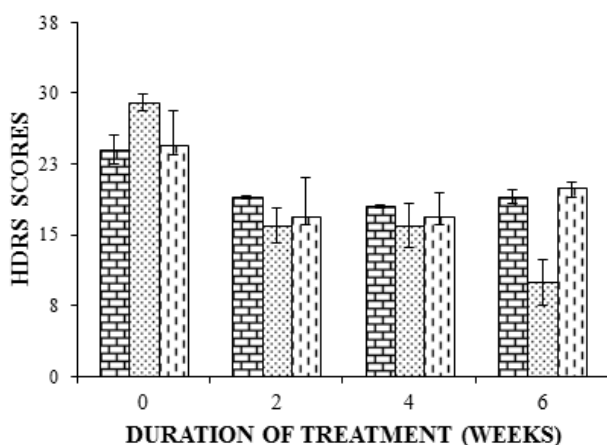
Fig. 2: Representative chromatograms of plasma samples of normal and depressed individuals.

High performance liquid chromatography

Levels of dopamine DA, DOPAC, HVA, 5-HT and 5-HIAA in all human plasma samples were determined using high performance liquid chromatography (HPLC, prominence 20A, Shimadzu, Japan) with electrochemical detection (L-ECD-6A, Shimadzu, Japan).

The aqueous mobile phase was prepared according to Curet *et al.* (1995) with slight modification, containing sodium phosphate monobasic (0.1M), EDTA.2Na (1 mM), 1-octane sulfonic acid (2.5mM) and acetonitrile (10%) in de-ionized de-gassed water (1L) and adjusted to pH 3.4 with phosphoric acid. The mobile phase was filtered through membrane filter (0.22 μm , Millipore, Ireland) and delivered by a pump (LC-20AT) at a flow

rate of 1 ml/min into pre-packed reversed phase HPLC column (C18 250 mm x 4.6 mm nucleosil column with particle size of 100-5 μm) at a ECD potential of +0.75 V. The retention time was used to identify the respective peaks after alignment with appropriate references (DA, DOPAC, HVA, 5-HT and 5-HIAA) using the specific software (LC solution). The concentrations were deliberated using standard curves obtained for all the individual standards.



Hamilton depression rating scale (HDRS) scores (mean ± s.e) before (0 week) and during 2, 4 and 6 weeks treatment in drug naïve depressed individuals with 20 mg/d fluoxetine (■, n = 7), paroxetine (▤, n=7) and 20 mg/d citalopram or 100 mg/d sertraline (▥, n=6). Significant differences in decline of scores are represented as **p*<0.05 and ****p*<0.005

Fig. 3: Effect of antidepressants on Hamilton depression rating scale scores during treatment.

Assay for monoamine oxidase-B enzyme activity in platelets

Monoamine oxidase-B (MAO-B) activity was determined fluorometrically (Dar *et al.*, 2005) using benzylamine (500μM) most selective substrate. Platelets (20μl), substrate (50μl) and buffer (130μl) were incubated at 37°C for 20min. The intensity of the adrenolutine fluorescence (adrenaline/peroxidase system) was determined spectrofluorometrically (RF-1501, Shimadzu, Japan) at an excitation and emission wavelength 405nm and 550nm. The MAO-B enzyme activity was expressed as nmoles of H₂O₂ in 10⁷platelets/h.

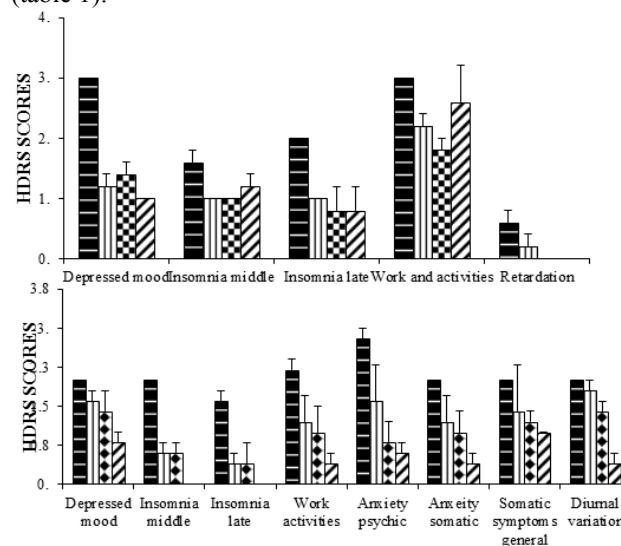
STATISTICAL ANALYSIS

The means were compared by SPSS software (version 12.0) using various statistical tests: The Student’s *t*-test, one way analysis of variance (ANOVA) followed by post hoc analysis through least significant difference (LSD) test. To identify the differences in frequency and severity between genders the χ²-test was also employed. The results were considered to be significant at the 5% level.

RESULTS

Severity of the depressive symptoms

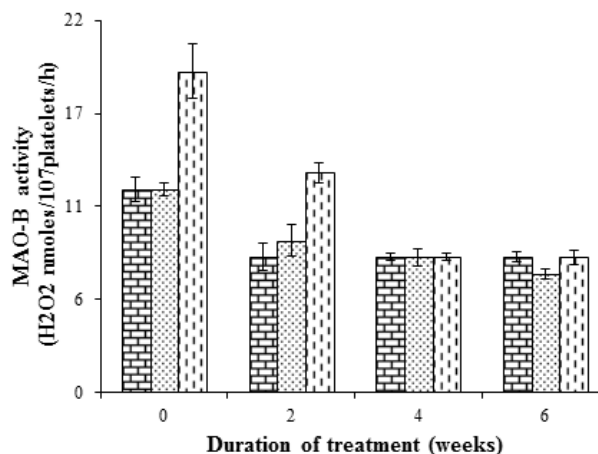
Considering the HDRS scores 20%, 24% and 55% of males suffered from mild, moderate and severe depression. However, in females severe depression was predominant (89%) and mild depression was not observed (table 1).



SYMPTOMS OF HDRS DURING TREATMENT WEEKS

Hamilton depression rating scale (HDRS) scores of individual symptoms of depression before (□, 0 week) and during 2 (▨), 4 (▩) and 6 (▪) weeks treatment in drug naïve depressed individuals (n=7 each group) with 20 mg/d a) fluoxetine and b) paroxetine. Significant differences in decline of scores are represented as **p*<0.05, ***p*<0.01 and ****p*<0.005

Fig. 4: Effect of fluoxetine and paroxetine on individual symptom scores using Hamilton depression rating scale.



MAO-B activity (nmoles/10⁷ platelets/h), before (0 week) and during 2, 4 and 6 weeks treatment in drug naïve depressed individuals with 20 mg/d fluoxetine (■, n = 7), paroxetine (▤, n = 7) and 20 mg/d citalopram or 100 mg/d sertraline (▥, n = 6). Significant differences in decline of scores are represented as **p*<0.05

Fig. 5: Effect of antidepressants on MAO-B activities during treatment.

In females the mean score of HDRS (25.1 ± 1.1) was (1.3x) higher than males (20.0 ± 1.0) (table 2). The individual symptoms scores including depressed mood, anxiety psychic, anxiety somatic, work and activities, agitation, somatic symptoms gastrointestinal and general were ~2x severe in females.

Frequency of depressive symptoms

The most frequent (90%) symptoms were depressed mood and anxiety psychic while, least frequent (~30%) symptoms were feelings of guilt and insight. The anxiety somatic, work and activities, insomnia early, somatic symptoms general and diurnal variation were evident in ~80%; insomnia middle, insomnia late, agitation, somatic symptoms general and weight loss in ~60%; and suicide, retardation, somatic symptoms gastrointestinal tract and hypochondriasis in ~50%. According to frequency in descending order, the three most frequent symptoms in males were depressed mood, anxiety psychic and insomnia early. However, in severely depressed females, most frequent symptom was anxiety psychic followed by depressed mood, anxiety somatic, diurnal variation with similar frequencies and third most frequent symptom was work and activities. The symptoms of insomnia middle, anxiety somatic and somatic symptom gastrointestinal were significantly more frequent in females. Though the frequencies of weight loss, hypochondriasis and genital symptoms were higher in males as compared to females but these differences were non-significant (fig. 1).

Levels of monoamines in normal and depressed individuals

The plasma levels (ng/ml) of monoamines and their metabolites in normal individuals were: DA (1.5 ± 0.2), DOPAC (3.6 ± 0.7), HVA (6.9 ± 0.4), 5-HT (2.6 ± 0.3), 5-HIAA (7.7 ± 0.4). However in drug naïve depressed the plasma levels (ng/ml) in males and females were: DA (2.1 ± 0.5 and 0.9 ± 0.3), HVA (9.2 ± 1.4 and 10.5 ± 1.4), DOPAC (2.7 ± 0.9 and 2.0 ± 0.6), 5-HT (5.2 ± 1.0 and 1.3 ± 0.5), 5-HIAA (11.7 ± 1.9 and 13.8 ± 2.0) (table 3), shown in representative chromatograms (fig. 2). The levels of HVA and 5-HIAA were significantly higher 43% and 66% respectively in depressed than normal individuals.

MAO-B activity in normal and depressed individuals

In normal individuals platelet MAO-B activities was significantly less (25%) than depressed individuals (14.1 ± 0.9 nmoles/ 10^7 platelets/h) (table 3), as there was no significant difference between males and females therefore gender based analysis not performed and data was pooled.

Effect of antidepressant drugs treatment

After 2 weeks of treatment, a significant decline (19%) in HDRS scores from 23.8 ± 1.1 to 19.2 ± 0.3 was evident in

fluoxetine treated group, with no further change till 6 weeks. However, individuals on paroxetine treatment showed a significant decline (40%) from 27.4 ± 0.8 to 16.4 ± 1.3 in scores after 2 weeks with a further reduction of 62% after 6 weeks of treatment. The third group treated with either sertraline or citalopram, (2 patients were dropped out and follow up was completed with 4 patients) also demonstrated a non-significant reduction in HDRS scores (31%) after 2 weeks of treatment was evident from 24.6 ± 2.3 to 17.0 ± 2.5 (fig. 3).

Regarding individual symptoms of depression, both fluoxetine and paroxetine induced a significant reduction of similar magnitude (60%) of the scores for depressed mood after 2 and 6 weeks, respectively. Furthermore, a significant lowering in the scores of insomnia (middle and late) was also evident after 2 weeks of treatment with both the drugs while paroxetine ($p < 0.005$) was more effective. Paroxetine also showed a significant decline in the scores of other symptoms including agitation (50%) and insomnia (70 to 85%). However, anxiety psychic, work and activities, diurnal variation and somatic symptoms general were reduced (30 to 70%) while anxiety somatic (80%) were attenuated after 4 and 6 weeks of treatment, respectively (fig. 4).

The platelet MAO-B activity, after 2 and 4 weeks of paroxetine treatment caused a significant decline from 11.7 ± 0.6 to 9.4 ± 0.9 (20%) that further declined to 6.8 ± 0.2 (43%), after 6 weeks. Although, similar pattern was also demonstrated with other SSRIs used but the further decline was non-significant (fig. 5).

DISCUSSION

Generally, depression is clinically recognized by a triad-symptoms including depressed mood, feelings of guilt and suicidal thoughts (Hamilton, 1960; Jang *et al.*, 2011). However, in the present study conducted on a sub-set of population from Karachi, the most frequent symptoms were depressed mood and anxiety whereas, suicidal thoughts were observed only in ~50% while feelings of guilt was least frequent. A similar pattern in depressed patients from other countries such as Thailand (Lotrakul *et al.*, 1996), Sri Lanka (Ball *et al.*, 2010) and England (Hamilton, 1989) has also been reported. In case of Pakistan, depressed mood and anxiety could probably be related to various situations in particular insecurity, terrorism, violence along with socioeconomic conditions, low literacy rate and uncertain political conditions (Mirza and Jenkins, 2004; Husain *et al.*, 2004). Feelings of guilt (30%) among Pakistani depressed patients are similar to the findings from Sri Lanka and Thailand, whereas, it was 30 to 40% higher in English and Koreans (Hamilton, 1989; Kim and Cho, 1993) populations, apparently due to differences in the social set-up of these societies. Notably, the low frequencies of suicide and feelings of guilt among

Pakistanis might be attributed to firm religious values that encourage absolute faith in Allah Subhana Tallah (God), negation of self-harm (in Islam suicide is non-permissible (haram)), strong social bonding with family and friends. Furthermore, the symptoms of work and activities emerged as a third most frequent symptom, while it is most frequent in Korea and England (Hamilton, 1989; Jang *et al.*, 2011). Pattern of other depression symptoms are generally in accordance to the studies from different Asian and Western countries with minor differences (Kim, 1977; Hamilton, 1989; Lotrakul *et al.*, 1996; Ball *et al.*, 2010) implying that similarities in the depressive symptoms do exist globally among different populations.

Table 1: Severity of depression in depressed males and females

| Severity of depression | Males | Females |
|------------------------|------------|------------|
| Mild | 9 (20%) | 0 |
| Moderate | 11 (24.4%) | 2 (11.1%) |
| Severe | 25 (55.5%) | 16 (88.8%) |

Values within parenthesis are percentage of total number of males (n=45) and females (n=18)

Severity of depression assessed according to Hamilton depression rating scale scores: Mild (10-13), Moderate, (14-18) and Severe (>19)

It is noteworthy, that in our study ~88% of females were severely depressed that is clearly reflected in the marked differences in the magnitude of the symptoms as compared to male counterparts that included all the three groups (mild, moderate and severely depressed). The absence of mildly depressed and 11% of the moderately depressed females in our study may be an estimation which are more likely due to the possibilities to overcome their problems by sharing it with their family members and close friends or hesitation on their part or their family to contact the psychiatrists. However, when the symptoms of depression become more apparent, accompanied by unusual behaviours and their inability to carry out routine domestic responsibilities only then they are taken to the clinicians.

Despite the variable depression symptoms among Pakistani males and females as mentioned above, differences between them were evident as the frequencies of depressive symptoms like insomnia middle, somatic symptoms gastrointestinal and anxiety somatic were significantly (32 to 83%) higher in females. These findings emphasizes that regardless of cultural and religious differences females are consistently more prone to depression across the globe as evident in Americans (Bennett *et al.*, 2005), Swiss (Ernst and Angst, 1992) and Indian (Patel, 2001) populations. Likewise, in our study, severity of depressive symptoms measured by HDRS scores was also 25% greater in females (25.1±1.1) as compared to males further supporting that they are more

susceptible to the causes leading to depression. Considering individual depressive symptoms, scores of depressed mood, anxiety psychic and somatic, agitation, work and activities, somatic symptoms gastrointestinal and general were significantly higher (30 to 100%) in females. Similarly, ~10% higher severity in American females has also been observed (Williams *et al.*, 1995). The increased susceptibility of females to undergo depression is probably associated with multiple risk factors such as low socio-economic status, life style, incidence of domestic problems, educational and occupational discriminations which are more pronounced in South Asian countries (Mumford *et al.*, 1997; Rahman *et al.*, 2009). Moreover, in females the contribution of other physiological factor(s) particularly hormonal changes cannot be ruled out (Patel *et al.*, 2006).

Besides environmental factors, the biological aspects of depression have been explained through altered plasma monoamine levels and MAO-B activities. Among normal Pakistani individuals, the levels of 5-HT (2.6 ± 0.3 ng/ml) and its metabolite 5-HIAA (7.7 ± 0.4 ng/ml) were either 2x to 7x lower (Nagaoka *et al.*, 1997; Mitani *et al.*, 2006) or 4x higher (Nagaoka *et al.*, 1997; Lee, 2000) than Japanese population. Likewise, levels of DA and its metabolite HVA and DOPAC were also either similar, or lower (1.9x) or higher (2x to 7x) (Minegishi and Ishizaki, 1984; Nagaoka *et al.*, 1997; Mitani *et al.*, 2006). These variations in levels of monoamine and their metabolites may be associated to genetic differences among them however, different techniques used in their determination and sample preparation cannot be ruled out. Our studies further demonstrated that the plasma levels of HVA and 5-HIAA were 1.4x and 1.7x significantly higher in depressed as compared to normal individuals. On the contrary, these levels were either similar (Devanand *et al.*, 1993) or lower in depressed subjects (Mitani *et al.*, 2006). The levels of DOPAC, HVA and 5-HIAA were 2.2x, 2.4x and 6.4x higher, respectively in Pakistani depressed individuals than in Japanese depressed individuals (Mitani *et al.*, 2006). Thus it is crucial to generate a global database from different countries and ethnic groups for endogenous neurotransmitters and their metabolite(s) levels involved in the depression (mild, moderate and severe). It is important to note that in contradiction to popular monoamine basis of depression, our data revealed non-significant alterations in 5-HT and dopamine levels in depressed subjects as compared to control. There are several reports in the literature supporting our observations particularly the monoamine depletion was shown to have no effect on the mood of healthy and depressed subjects (Berman *et al.*, 2002). Furthermore, the antidepressant action was shown to be induced by inhibitors as well as enhancers of serotonin reuptake induced (Mennini *et al.*, 1987; Uzbay, 2008) and were also effective via diminishing serotonergic neurotransmission (Ogren *et al.*, 1979). These conflicting

Table 2: Severity of Hamilton depression rating scale symptoms in drug naïve depressed males and females

| S. No | Symptoms | Range of scoring | Mean scores of individual symptoms | |
|-------|----------------------------------------|------------------|------------------------------------|------------------|
| | | | Males (n = 45) | Females (n = 18) |
| 1 | Depressed mood | 0-4 | 1.8 ± 0.1 | 2.5 ± 0.2* |
| 2 | Feelings of guilt | | 0.6 ± 0.1 | 0.5 ± 0.1 |
| 3 | Suicide | | 0.9 ± 0.1 | 1.1 ± 0.3 |
| 4 | Insomnia early | 0-2 | 1.5 ± 0.1 | 1.4 ± 0.2 |
| 5 | Insomnia middle | | 1.0 ± 0.2 | 1.4 ± 0.2 |
| 6 | Insomnia late | | 0.8 ± 0.1 | 1.2 ± 0.2 |
| 7 | Work and activities | 0-4 | 1.9 ± 0.2 | 2.6 ± 0.2* |
| 8 | Retardation | | 0.6 ± 0.1 | 0.6 ± 0.1 |
| 9 | Agitation | | 0.8 ± 0.1 | 1.6 ± 0.1** |
| 10 | Anxiety psychic | | 2.1 ± 0.2 | 2.8 ± 0.1* |
| 11 | Anxiety somatic | | 1.2 ± 0.1 | 1.9 ± 0.1** |
| 12 | Somatic symptoms gastrointestinal | | 0.5 ± 0.1 | 1.0 ± 0.1** |
| 13 | Somatic symptoms general | 0-2 | 0.9 ± 0.1 | 1.3 ± 0.2* |
| 14 | Genital symptoms | | 0.6 ± 0.1 | 0.3 ± 0.1 |
| 15 | Hypochondriasis | 0-4 | 0.9 ± 0.1 | 0.4 ± 0.1 |
| 16 | Loss of weight | 0-3 | 0.9 ± 0.1 | 0.6 ± 0.1 |
| 17 | Insight | 0-2 | 0.7 ± 0.1 | 0.5 ± 0.2 |
| 18 | Diurnal variation | 0-2 | 1.2 ± 0.1 | 1.5 ± 0.1 |
| | Mean summation of HDRS symptoms (1-18) | | 18.9 ± 0.5 | 23.2 ± 0.6 |
| | HDRS total scores | | 20.0 ± 1.0 | 25.1 ± 1.1** |

HDRS = Hamilton Depression Rating Scale, * $p < 0.05$ and ** $p < 0.01$ significantly higher than males

Table 3: Levels of monoamines and their metabolites in plasma and monoamine oxidase-B activity in platelets of normal and drug naïve depressed individuals

| | Normal individuals | Depressed individuals |
|--------------------|--------------------|-----------------------|
| No. of individuals | 52 | 20 |
| Age (years) | 30.7 ± 1.4 | 34.0 ± 2.4 |
| DA | 1.5 ± 0.2 | 1.6 ± 0.4 |
| DOPAC | 3.6 ± 0.7 | 2.2 ± 0.5 |
| HVA | 6.9 ± 0.4 | 9.9 ± 1.0*** |
| 5-HT | 2.6 ± 0.3 | 2.6 ± 0.9 |
| 5-HIAA | 7.7 ± 0.4 | 12.8 ± 1.4*** |
| MAO-B | 11.3 ± 0.5 | 14.1 ± 0.9*** |

Values (mean ± s.e) represent concentrations (ng/ml) of monoamines and their metabolites: dopamine (DA), dihydroxyphenylacetic acid (DOPAC), 5-homovanillic acid (HVA), hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in platelet poor plasma using HPLC. For experimental details refer to fig. 2.

Monoamine oxidase-B (MAO-B) activity in platelets of normal (n=52) and drug naïve depressed (n=20) individuals was determined fluorometrically using benzyl amine (500 µM) as substrate and expressed as H₂O₂ nmoles /10⁷platelets/h. Excitation wavelength = 405 nm, emission wavelength = 550 nm

Significant differences are represented as: *** $p < 0.005$

patterns are more likely due to differences in drug metabolizing enzymes emphasizing the importance of pharmacogenetics and the genetic make-up of various populations.

Furthermore, in depressed patients the low levels of 5-HIAA, HVA and MAO-B activity in cerebrospinal fluid, platelets and plasma has been linked with suicidal thoughts and its attempt (Spreux-Varoquaux *et al.*, 2001). Hence, in present study, the high levels of aforementioned metabolites support the lower frequency and scores of suicidal thoughts in depressed subjects. It is strengthened

further with the reported rate of suicides in Pakistan i.e. only 1.2% (Human rights commission, 2012) as compared to 10 fold higher rates in Japan (Nikkei, 2013).

In our study, the platelet MAO-B activity in normal individuals (11.3±0.5 nmoles/10⁷platelets/h) was greater (1.2x) compared to Americans (Lenders *et al.*, 1996) and Italians (Coccini *et al.*, 2002) thereby, highlighting the importance of population-based studies. The MAO-B activity in our depressed patients was significantly higher (20%) than normal individuals, in line of the findings by other investigators (Reveley *et al.*, 1981). On the other

hand, decreased activities in depressed subjects have also been noted (Alexopoulos *et al.*, 1987). Such conflicting results are probably due to the type of depression cases. For example, unipolar patient demonstrated higher enzymic levels whereas; bipolar patients frequently reported lower values (Sandler *et al.*, 1981). Keeping this in mind the depressed individuals in the present study probably belongs to unipolar group, showing higher enzyme activities as compared to normal subjects.

It is well established that clinical trials on antidepressant drugs improve the symptoms of severe depression (Fournier *et al.*, 2010). However, the treatment is mostly effective after 6 weeks (Richelson, 2001), but earlier (before 2 weeks) clinical improvement has also been reported (Szegedi *et al.*, 2003). In present study, the severely depressed drug naïve patients selected for treatment, had mean HDRS scores of 25.1 ± 1.1 which significantly declined (>20%) just after two weeks of treatment with SSRIs (fluoxetine, paroxetine and citalopram and sertraline). Similar magnitude of reduction in HDRS (Seemullar *et al.*, 2008) and MADRS (Montgomery-Asberg Depression Rating Scale) was also evident after 2 weeks of treatment (Moller *et al.*, 2007). Furthermore, the contention regarding effectiveness of antidepressant drugs in case of severe depression (Fournier *et al.*, 2010) is also supported by our observations as both fluoxetine and paroxetine induced a significant decline (19% and 40%, respectively) in HDRS after 2 weeks while later was not only 2x better but also continued to induce further decline (62%) after 6 weeks of treatment. On the contrary, in severely depressed subjects both antidepressants induced similar effect as identified by either HDRS rating scale (Fava *et al.*, 1998) or MADRS (Tignol, 1993). This discrepancy in the responses of individuals to the drugs could possibly be due to the genetic diversity in drug metabolizing enzymes emphasizing the importance of pharmacogenetics in the treatment of depression (Steimer *et al.*, 2001). Considering the effect of treatment on the symptom of depressed mood, fluoxetine appeared to be more effective eliciting 60% decline as compared to paroxetine, response observable after 6 weeks. Whereas, after 2 weeks of treatment the paroxetine effect was more pronounced on other symptoms causing significant reduction in the severity of insomnia early (85%), middle (65%) and late (75%). An improvement in sleep disturbances after 4 weeks of paroxetine treatment has also been reported earlier (Ontiveros and Garcia-Barriga, 1997). A significant improvement (55 to 85%) in other symptoms (anxiety psychic, work and activities, diurnal variation and somatic symptoms general) after 4 weeks of treatment with paroxetine also showed sustained response with probable remission. These results suggest that in our population paroxetine is most effective in the improvement of psychological and somatic symptoms of patients.

Despite of improvement in the depressive symptoms clinically, the levels of the monoamines measured in our investigation remained unchanged after 6 weeks of treatment is in accordance with earlier report (Saldanha *et al.*, 2009), where serotonin levels also remained unchanged after 6 months of treatment with SSRIs. Antidepressant drugs were shown earlier to reduce MAO-B activity (Fowler *et al.*, 1982), while fluoxetine inhibits both MAO-A and -B (Mukherjee and Yang, 1997 and 1999), thereby providing a possible reason for unchanged monoamines levels during first six weeks of treatment in our study.

It has emerged that high platelet MAO-B activities and HDRS scores in drug naïve depressed subjects could serve as indicators for appropriate antidepressant treatment response. Although in these individuals direct relationship between HDRS scores and MAO-B activity was not evident but concomitant decline after treatment implies that platelet MAO-B activity in effectiveness of drug and clinical improvement cannot be undermined and maybe useful for early detection of drug response. However, investigation with larger data is needed to reach a definite conclusion.

CONCLUSION

In conclusion, three most frequent symptoms in a sub-set of depressed patients from Karachi, Pakistan, were depressed mood, anxiety psychic and work and activities whereas, the symptoms under cultural influence like feelings of guilt and suicidal thoughts were less frequent. In females symptoms of depression were more frequent and severe as compared to males. High values of plasma HVA and 5-HIAA and platelet MAO-B activity in these patients are accountable for lower risk of suicides in our population. After treatment, an early decline in HDRS is an indicative of rapid response of patients towards commonly prescribed SSRIs with paroxetine being more effective against insomnia and somatic symptoms. The simultaneous decline in MAO-B activity further supports their role in clinical improvement.

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