Oxidative Stress and Depression among Male Shift Workers in Shahid Tondgouyan Refinery

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Objective: The aim of this study was to determine the oxidative stress; serum level of Total Antioxidant Capacity (TAC)and Malondialdehyde (MDA) level and the depression score among the depressed rotational shift workers in Shahid Tondgouyan Refinery in Tehran (Iran).
Methods: A cross-sectional study was conducted among all the 189 shift workers in Shahid Tondgouyan oil refinery who were eligible to participate in the study. They did not take any antidepressants for two months or any supplements for two weeks prior to the study entry. Written consent was obtained from the participants. 21- Item Beck Depression Inventory was used to measure the depression level. Furthermore, body weight, height and systolic and diastolic blood pressure were collected from all the participants. The levels of Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA) were measured by 8 ml fasting blood sample. MDA was determined by thiobarbituric acid reaction. Serum total antioxidants were measured using the spectrophotometric ABTS. In the ABTS test, 2,2'-azinobis (3-ethylbenzthiazoline-6-acid) (ABTS) is converted into its radical cation (ABTS•+) by addition of sodium persulphate. This blue-green radical cation absorbs light at 734 nm. ABTS•+ is reactive towards most antioxidants. Descriptive statistics, ANOVA, ANCOVA and regression tests and correlation were used to analyze the data using SPSS software version 16.
Results: The age of the participants ranged from 21 to 52 years. The mean age of the participants was 30.58 year (±6.97yr). Of all the participants, 28% (n= 53) had no depression symptoms (depression score between 0 and 9), 65.1% (n=123) were categorized as having mild depression (depression score between 10 and 18) and 6.9% (n=13) were categorized as having moderate depression (depression score between 19 and 29). The participants' BMI ranged from 15.9 to 34.3; the mean BMI of the participants was 24.82 kg/m^2 (+ 3.81 kg/m^2). The mean of the serum TAC level was 2.51 (± 0.56) mg/dl, and the mean serum MDA level was 3.67(± 1.08) µmol/l. There was a significant difference in the mean TAC concentration between the non-depressed group and the group with mild depression (p=0.029).
Conclusion: Depression was associated with reduced mean TAC concentration and an increase in MDA level. There was a linear relationship between the depression score and shift work experience among the rotational shift workers, which showed a high level of stress and depression among the shift-workers.

Keywords: *Depression, oxidative stress, Malondialdehyde, total antioxidant capacity, shift work*

Iran J Psychiatry 2014; 9:2: 76-82

Shift work is defined as a work schedule outside of the normal daytime working hours (7 am to 6 pm) (1). The trend in our society is toward an increase in shift work and it is essential for many industries, such as refineries to have a 24 hour work pattern (2). More than 20 to 30 percent of workers are shift workers (3). Shift work can alter human circadian system which is normally synchronized with the solar day and as a result the pattern of sleep-wake becomes misaligned (4). Circadian system is completely influenced by ambient light (5). Shift workers can never be adapted to their sleep/activity cycle, which is necessary for their shift work (6).

Disruption of normal circadian system can cause physiological and psychological problems and shift work negatively impacts workers' health condition (7) and causes diseases resulting in absenteeism from work. Among the health problems caused by shift work is sleep disorder (8). Studies in other countries showed that the prevalence of difficulty initiating sleep is higher in rotational shift workers compared to regular day workers (9).

Based on different studies abroad, shift workers complain of sleep disorder and insufficient sleep,

ranging from 10% to 90 % (10-12). Furthermore, sleep deprivation and occupational stress lead to more sleepiness and reduce the neurobehavioral function, and therefore increase the risk of depression (13).

There is an association between poor sleep and symptoms of deep depression in male shift workers (14). Depressed patients show increased oxidative stress (15). In addition, sleep deprivation reported to cause oxidative stress, resulting in the formation of Reactive Oxygen Species (ROS) and eventually leads to neuronal and cellular damage. In the human body, ROS are formed in the cytosol, mitochondria, lysosomes, peroxisomes and plasma membranes under both physiological and pathological conditions (16); and their levels can be increased by different stressful situations such as occupational stress (17-18). Stressful conditions lead to the formation of excessive ROS and cause oxidative stress (19). Oxidative stresses occur when the production of free radicals exceeds the defensive response of the antioxidant system. Oxidative stress has a major role in the causality of some disorders that have higher prevalence in shift workers (20). Malondialdehyde (MDA) increases in body during excessive oxidative stress (21).

Lipid peroxidation is one of the major outcomes of free radical-mediated injury that directly damages membranes and generates a number of secondary products including aldehydes such as MDA, which is the most abundant individual aldehyde, resulting from lipid per oxidation (22). Also, total antioxidant capacity (TAC) decreases in oxidative stress (23). Free radicals initiate a cascade, causing lipid peroxidation, DNA damage, cell death and neurological problems. Total plasma antioxidant capacity is measured as an indicator of oxidative stress (24).

In Iran, few studies have been carried out about shift workers (25-26). Most of these studies are concerned with shift workers in Iranian hospitals. Studies about the shift workers in Iranian industries are rare (27). The results of these few studies have revealed that Iranian shift workers are at risk of depression (28). In the study of Zamanian and colleagues, it was shown that the shift work hospitals security had a significant higher level of mental disorders compared to the control group (non-shift workers). In a study on nurses in a hospital in Shiraz, it was shown that the mental disorders are higher among shift workers compared to the day workers (29-30). Dehghani and colleagues in a study on the nurses (84% of the nurses were shift workers) showed that 58.8% were suffering from depression in different degrees (31).

This study was conducted to determine the level of depression and oxidative stress by measuring TAC and MDA among rotational shift workers in Shahid Tondgouyan Tehran Oil Refinery.

Material and Methods

Research Participants

All the shift workers in Tehran Shahid Tondgoyan Oil refinery who met the inclusion criteria and consent to participate in the study were included (189 out of 456). Of the 456 potentially eligible candidates in Tehran Shahid Tondgoyan Oil refinery were screened for depression symptoms using 21- item Beck depression inventory questionnaire. A total of 397(87.06%) workers returned the questionnaire. Out of the 397 workers, 208 workers did not meet the inclusion criteria. Finally, 189 shift workers aged 21-52 were enrolled in the study. It should be noted that all of the shift workers in Tehran Shahid Tondgoyan Oil refinery were male; and they had to work 8 hours per work shift. Their work hours were as follows: four night shifts (night-morning), three days off, four afternoon shifts and one day off. Inclusion and exclusion criteria: The study inclusion criteria were giving written consent to participate in the study and wash-out periods of two months for antidepressants medicines and two weeks for supplements prior to the study entry .

The exclusion criteria were history of thyroid diseases, liver diseases, kidney diseases, diabetes, cardiovascular diseases, cancer, hypertension (based on physical examination), being a professional sportsman, smoking, consuming alcohol and substance abuse. Information was collected using a self-administered general questionnaire.

Data Collection Tools

Data was collected using a self-administered general questionnaire "21- item Beck depression inventory" (32), which was translated into Persian by Dr. Kaviani in 2002 (33). The validity and reliability of the Persian version were assessed by Dabson and Mohammadkhani in 2007. They found a test- retest reliability of more than 0.9, measured by Cronbach's alpha and a high level of validity measured by factorial analysis and content analysis (34). The Beck Depression Inventory Second Edition (BDI-II) is a 21-item self-report instrument intended to assess the existence and severity of symptoms of depression as listed in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV; 1994). BDI assesses depressive symptoms and has been reported to be highly reliable regardless of the population. It has a high coefficient alpha, (0.80), its construct validity has been established and it is able to differentiate depressed from non-depressed patients (32). The general questionnaire elicited information on age, marital status, work experience, shift work experience, education, sports, smoking, alcoholic drinks, narcotics and drugs. For the enrolled participants, weight and height were measured with Seca standard tools (Germany) with 0.1 cm and 100 g precision, while the participants had the least clothes on and were barefoot; and their body mass index (BMI) was calculated from the formula [weight(kg)/high(m)^2]. Diastolic and systolic blood pressures were measured using a mercury sphygmomanometer with 5

mmHg precision from the right arm and after 10 minutes of resting in the sitting position. A history of thyroid diseases, liver diseases, kidney diseases, diabetes, cardiovascular diseases, cancer and hypertension was collected based on physical examination performed by the interviewing physician in the health center of Tehran Shahid Tondgoyan Oil Refinery.

Blood Sample Collection: To measure the level of TAC and MDA, 8 ml blood sample was collected from the vein anterior to the elbow at the sitting position and after fasting for 10-12 hours at 7.30 am to 8.30 am. Needle holder 21 in a gel-containing tube without anti-coagulant (Behdarou Company) was used. Then, the serum was separated using centrifuge for about 10 minutes at 1500 rpm. The extracted serum was transferred into the micro tubes which were labeled with identifier code and was kept at -70 °C until the time of the analysis. The serum MDA level was determined using the method described by Satoh (35). In this method, MDA was determined by thiobarbituric acid (TBA) reaction and separation on HPLC. UV detection was performed at 532 nm.

Serum total antioxidants was measured using the ABTS) 2, 2 - Azino-bise sulfonic acid). This method is based on the reconstruct cation ABTS (the maximum absorbance at wavelengths 820, 734 and 660 nm) and the chain-breaking antioxidants are low molecular mass. In the ABTS test, 2,2'-azinobis (3-ethylbenzthiazoline-6-acid) (ABTS) is converted into its radical cation (ABTS•+) by addition of sodium persulphate. This blue-green radical cation absorbs light at 734 nm. ABTS•+ is reactive towards most antioxidants (36).

As the type of work of the shift workers was similar in this study, just work history and shift work history were considered as independent variables .

TAC was measured in Dept. of Biochemistry, Tehran University of Medical Sciences, and MDA was measured in Tehran Nour Research Center .

Statistical Analyses

All analyses were performed using the statistical package for social sciences (SPSS) version 19.0 for windows (IBM Corporation, New York, United States). Descriptive statistics were shown as mean and standard deviation. The relationship between the quantitative variables was tested by bivariate analysis. The difference between groups was analyzed using ANOVA, ANCOVA, general linear regression and correlation test. The significant level was set at $P<0.05$.

Ethical Consideration

Review Board of Tarbiat Modares University approved the study. Ethical approval was obtained from the Medical Ethics Committee of Tarbiat Modares University in Tehran-Iran. All the participants gave written consent to participate in the study. The participants were assured that the data were kept confidential and their identity would not be revealed and the data would not be used except for the research purposes.

Result

Table 1 demonstrates the demographic characteristics and clinical examination information of the participants. All the participants were male with age range of 21 to 52 years. The mean age of the participants was 30.58 year (6.97yr) and most of the participants were in the age group of less than 30 years (n=120, 63.5%). The educational level of the most participants was diploma (n=114, 60.3%), the educational level of 37% (n = 70) of the participants was higher than diploma. Regarding marital status, 64.6% of the participants (n = 122) were married. With regards to the type of work, 81% of the participants (n = 150) were operational workers and 19% (n = 36) were firefighters. The work experience of 63.0% (n = 119) of the participants was less than 6 years, 22.8% (n = 43) were employed between 6 and 15 years, 12.7% (n = 24) were employed between16 and 25 years and 1.6% (n = 3) were employed for more than 25 years. Shift work experience in 70.4% (n = 133) of the cases was less than 6 years and for 11.6% (n=22) of the cases was between 16 and 25 years .

Depression score ranged from 0 to 29 in the participants. The mean depression score was 11.33(±5.61). Of all the participants, 28% (n=53) were without any depression symptoms (depression score between 0 and 9), and 6.9% (n = 13) were categorized as having average depression (depression score between 19 and 29). The participants' BMI ranged from 15.9 to 34.3; the mean BMI of the participants was 24.82kg/ m2 (+ 3.81 kg /m^2). The mean of the serum TAC level and MDA level is demonstrated in Table 1

Table 2 shows the correlation between age, BMI and shift work experience with the depression score, TAC and MDA concentration. There was a significant correlation between depression score and shift work history (p = 0.011), but no correlation was observed between age and BMI with the depression score, TAC and MDA concentration. Also, no correlation was seen between shift work experience and TAC or MDA concentration.

The result of general linear regression test between shiftwork history and mean depression score is shown in Table 3.

There was a significant difference in the mean depression score between education groups ($p<0.024$) (ANOVA test). The Scheffe test was used to determine whether the depression score was significantly different between the different educational groups. The Scheffe analysis showed a significant difference in the mean depression score between diploma and higher than diploma educational level groups (p = 0.02). The mean depression scores in those participants with an academic degree of higher than diploma were more than those with diploma.

Table1: Demographic Characteristics and Clinical Examination Information of Participants

Demographic and clinical variable	
	Mean (SD)
Age (year)	30.58(6.970)
Work experience (year)	6.59(6.48)
Shift work experience(year)	5.82(6.06)
	Number (%)
Marital status	
Single(n=49)	67(35.4)
Married(n=87)	122(64.6)
Education	
Less than High school diploma	5(2.6)
Diploma	114(60.3)
More than High school diploma	70(37)
Clinical factors	**Mean (SD)**
BMI ($^{kg}/_{m^2}$)	24.82(3.81)
Diastolic Blood Pressure (mmHg)	66.61(10.70)
Systolic Blood Pressure (mmHg)	109.26(13.18)
Depression scores	11.33(5.61)
Total Antioxidant Capacity (mg/dl)	2.51(0.56)
Malonaldehyde (µmol/l)	3.67(1.08)

Table 2: Correlation between Age, Body Mass Index and Shift Work Experience and Depression Score, Total antioxidant capacity and Malondialdehyde Concentration

	Age (n=189)	**BMI (n=189)**	**Shift work experience (years) (n=189)**
Depression score	r=0.123 p=0.093	r=0.022 p=0.762	r=0.184 p=0.011*
Total antioxidant capacity g/dl	r=-0.013 p=0.858	r=-0.091 p=0.213	r=0.091 p=0.211
Malondialdehyde µmol/l	r=-0.088 p=0.229	r=0.055 p=0.454	r=-0.088 p=0.227

* Significant association

Table 3: Regression between shiftwork history and mean depression score

Variable	B	Beta	t	P value
Shiftwork history	0.170	0.184	2.55	0.011

a. Dependent Variable: Depression score

Table 4: The Relationship between Marital Status and Educational Level with Depression Score

Variable	Depression score	Total antioxidant capacity mg/dl	Malonaldehyde µmol/l
Marital status (n=189)			
Married (mean ± SD)	10.9±5.2	2.5± 0.65	2.5 ± 0.64
Single (mean ± SD)	11.5± 5.8	3.6±1.2	3.7 ± 1.01
P* value	0.461	0.532	0.995
Educational level (n=189)			
Primary high school (mean ± SD)	12.6 ± 8.5	2.3 ± 0.6	3.6 ± 1.0
Secondary high school (mean ± SD)	10.5 ± 5.5	2.5 ± 0.5	3.8 ± 1.1
Diploma (mean ± SD)	11.7 ± 4.7	2.5 ± 0.5	3.6 ± 1.0
Bachelor or above (mean ± SD)	13.2 ± 5.4	2.6 ± 0.6	3.5 ± 1.0
P# value	0.042	0.508	0.686

*t test
ANOVA test

Table 5: Association between marital status and educational level after adjustment for shiftwork history

Variable	Degree of freedom	F test	P* values
Shiftwork history	-	4.5	0.034
Marital status	1	0.212	0.646
Educational level	3	1.94	0.125

* ANCOVA test

Table 6: Biomarkers Concentration in Different Depression Groups among Shift Workers

Variable	Depression group			p Value
	≤ 9 (n=53)	10-18 (n=123)	19-29 (n=13)	
Total Antioxidant Capacity mg/dl	2.68 ± 0.60	2.44 ± 0.59	2.51 ± 0.55	0.038*
Malonaldehyde μmol/l	3.53 ± 1.04	3.76 ± 1.12	3.36 ± 0.71	0.24

This study showed no significant difference between the mean depression score among different marital status groups (p>0.05) (t-test) (Table 4).

ANCOVA test was used to adjust for the shiftwork history as a confounder for association between educational level and marital status and depression score. The result of ANCOVA test showed that after adjustment of r shiftwork history, no significant association was found between the depression score and marital status and educational level (Table 5).

Table 5 shows the relationship between TAC and MDA concentration and depression scores. There was a significant difference in the mean TAC concentration between the depression groups among the shift workers. The mean TAC concentration was 2.7mg/dl in the group without depression symptoms, 2.4 mg/dl in the group with mild depression and 2.5mg/dl in the moderately depressed group. The result of Scheffe test revealed a significant difference in the mean TAC concentration between the non-depressed group and the group with mild depression (p = 0.029). There was furthermore no, no significant difference was found in the mean MDA concentration between the different depression groups (p = 0.24) (Table 6).

Discussion

To our knowledge, the present study was the first in Iran to examine depression in shift workers and its association with oxidative stress markers (TAC and MDA as indicators of oxidative stress). This study was conducted in one of the most important industries in Iran, the refineries. In the present cross-sectional study, the association between age, BMI, shift work experience, marital status on depression score, total plasma TAC and MDA were assessed.

This study showed that the depression score was more among higher educational level group. This finding is in accordance with a study by Lin et al. which showed that the frequency of major depressive disorder (MDD) was higher among people with higher education (36). In the present study, the depression score had a positive relation with the shift work experience (r = 0.218, p<0.05). This result is in line with the result of the previous study by Scott et al. (37). However after adjustment for shiftwork history, no association was found between the mean depression score and educational level.

The results of this study did not show any correlation between BMI and TAC, MDA and depression score. However, some studies showed that TAC is inversely related to weight (24) and MDA concentration is higher in the obese patients (39). In some studies, BMI and depression were reported to be associated (40) and some studies found a weak inverse linear trend between obesity and depressive symptoms among males (41), but some other studies showed that association between BMI and depression is non-linear but U- shape for both genders (42).

This research did not show any correlation between age and TAC or MDA; however, the study of Sharifian et al. (24) found a correlation between age and total plasma antioxidant capacity through a borderline Pearson correlation. Nonetheless, in Sharifian's study, the confounding effect of BMI was not controlled and this borderline correlation might be related to the impact of BMI .

This study showed that TAC level was significantly lower in the mild depressed group compared to the non-depressed group. Some studies have reported a decreased level of TAC among depressed individuals (43-44). However, findings about the TAC level in depressed individuals are not consistent; for example, the study of Sofic et al. reported no change in the serum antioxidant capacity among depressed individuals (45).

In this study, no significant difference was found in the TAC level between mild depressed individuals and moderate depressed individuals, which may be a result of the low number of individuals in the moderate depression group and if the number was more, the difference could have been significant .

In this study, the mean MDA concentration was 3.67 (± 1.08) mol/l which is more than 5 times higher than the normal value for MDA that is less than 0.7 mol/l (46, 17). This finding shows that the oxidative stress among these shift-workers is much higher than the general population. This finding is consistent with the findings of some studies that reported major depression is associated with increased levels of serum MDA (47-48).

Study limitations: As the type of work of shift workers were similar in this study, only the limited number of variables including BMI, work history and shift work history were considered and other work related stressors were not investigated.

Conclusion

The mean TAC concentration was significantly lower among the mild depressed individuals compared to the non-depressed. The mean MDA serum level was higher than the reference range and there was a linier

relationship between the depression score and shift work experience among the rotational shift workers, which shows a high level of stress and depression among shift-workers.

Acknowledgment

This study is a part of a PhD thesis and is financially supported by Tarbiat Moddares University. The authors would like to thank Dr. Ramin Tofighi the Head of the Health Center, Dr. Mohamad Reza Yavari, Mr. Mahmoud Nikbakht the Head of the Research Center, Ahmad Kheiri the Head of the Operation Center and Ms. Ashraf Heidari the Head of the Occupational Health Center in Tehran Shahid Tondgoyan Oil Refinery, who sincerely helped us with this study.

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