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# Serum Pro-oxidant-Antioxidant Balance Assay in Nurses who Working Day and Rotating Night Shift

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## Abstract

**Background:** Nursing is a very stressful occupation, particularly when associated with changing shift working that may result in oxidative stress. Oxidative Stress is created by a misbalance between pro-oxidants and antioxidants towards pro-oxidants. Damage to lipids, protein, DNA, growth and cell function is obtained as result of oxidant. OS has been played an important role in many of diseases. In this study, we assessed the potential for OS by determining the pro-oxidant-antioxidant balance assay (PAB assay) in nurses who worked variable shift patterns; compared with nurses only working day shifts and a non-nurse control group.
**Methods:** Sera of 44 nurses with rotating shift working (night and day) and 43 nurses working day shift only and 80 healthy subjects, who did not work shifts and were not nurses, were collected and serum PAB was measured.
**Results:** A significant higher serum PAB value was observed in shift working and daytime nurses (152.28 $\pm$ 43.64 HK) in comparison to the control group (63.64 $\pm$ 34.41 HK index) P Value = 0.001. In day time nurses, also serum PAB between male (119.09 $\pm$ 47.14 HK) and female (162.53 $\pm$ 37.02 HK) in nursing workers was significant P value = 0.005. In the night shift working nurses, there was also a significant difference of PAB value between male (129.89 $\pm$ 35.76 HK) and female (170.46 $\pm$ 44.47 HK) P value = 0.002. Serum PAB between night shift workers (151.10 $\pm$ 45.02 HK) and daytime nurses (153.44 $\pm$ 42.66 HK) was no significant. A significant relationship was observed between serum PAB value and gender in nurses. There was no significant correlation between age and serum PAB value.
**Conclusions:** The serum pro-oxidant-antioxidant balance (PAB) assay may reflect oxidative stress in nurses. Females nurses may be exposed greater level of oxidative stress than male nurses. In our study shift working did not affect serum PAB levels, but the PAB levels were different in male and female groups.

*Keywords:* Serum Pro-Oxidant-Antioxidant Balance Assay, Oxidative Stress, Shift Working

## 1. Background

High rates of work-related stress are found in nurses in comparison with other occupations. Nurses are potentially subject of higher level of chronic stress, which can be emotionally draining, but may also have an impact on health (1). Some nurses work in day shifts and others in rotating day-night shifts cycles. Sleep deprivation and a stressful working environment may lead to physiological dysfunction, including vascular dysfunction (2) which can also effect on individual subsequent workdays. Rotating night shift workers might develop many problems related to health, as fatigue, sleep problems, anxiety and difficulties in maintaining regular lifestyles. Rotating shift work has also been related with increased risk of metabolic syndrome, diabetes, cardiovascular disease and cancer (3-7). Rotating shift work can also act as an oxidative stressor. Stress situations cause increase of free radicals and could product oxidative stress. Stress has been attending a basic factor in the etiology of many diseases (8).

There are the numerous pro-oxidants (POX) and antioxidants (AO), and it is important to preserve a balance between the production and the omission of POX. Oxidative stress (OS) is a misbalance between POX and AO in attention of POX. POX ($O_2^-$, $H_2O_2$, OH. etc.) obtain either from metabolic processes or from external sources and can potentially react with several important target molecules. AO thwart the over plus amount of the POX before they can harm these necessary molecules. AO defenses include of

water soluble antioxidants (vitamin C, urate, etc.), the lipid soluble antioxidants (vitamin E, A, etc.) and the enzymatic antioxidants (catalase, peroxidase, dismutase, etc.) (9).

Various methods exist to measure the concentration of oxidant and anti-oxidant separately. For example, Ochi and Cutler invented a diagnostic plot taken from the measurement of 82 assays which specify both the oxidative stress and the antioxidant profile (10). Nevertheless, the total influence of the pro-oxidant or the antioxidant molecules in serum is an additive or potentially synergistic and this may lead to an inaccurate assessment. Consequently, different methods have been developed in order to measure the whole oxidants (such as total oxidant status (TOS) and FOX assays) or antioxidants (such as the ferric reducing ability of plasma (FRAP) and oxygen radical absorbance capacity (ORAC) assays), singly. In this context, Pro-oxidant-antioxidant balace (PAB) assay is a method to measure the balance of oxidants and antioxidants simultaneously in one assay to give a measure of redox index status (9, 11, 12).

## 2. Objectives

The target of this study is to show the ability of pro-oxidant antioxidant balance assay in determining OS in this population in comparison to control group and also comparing the OS between shift-working and daytime nurses.

## 3. Methods

### 3.1. Chemicals for Assay

TMB powder (3, 3', 5, 5'-Tetramethylbenzidine, Fluka), peroxidase enzyme (Applichem: 230 U/mg, A3791, 0005, Darmstadt, Germany), chloramine T trihydrate (Applichem: A4331, Darmstadt, Germany), hydrogen peroxide (30%) (Merck). Molecular biology grade reagents were used and preparations were done in double distilled water.

### 3.2. Subjects

All of subjects had written informed consent. The case societies were nurses from different wards of hospitals of Mashhad University of Medical Sciences of Iran in 2013. 1 mL of serum was collected from 87 nurses working in hospitals, 44 who worked at rotating day-night shift (at least 13 night shift during the month with day shift) and 43 who worked at day shift only in general wards. Also we classified nurses into two sex groups, males and females, (None of the nurses and control group had no acute or chronic illness), and serum of 80 subjects who did not work-shifts nor in nursing, were healthy between April to June 2013. All groups consisted of non-smokers who did not consume alcohol and had no obvious clinical diseases. The study protocol was approved by the ethics committee for clinical research of the Mashhad University of Medical Sciences (MUMS).

### 3.3. Collection of Serum Samples

After taking samples from subjects, the samples were centrifuged at 1500 g for 15 minutes to separate serum from blood (we collected the samples from nurses during their work at hospital in day and night shifts separately and the samples got after a time off meal). And the serum of control group were prepare at BUAll Research Institute. Hemolytic samples were excluded from analysis. Serums were stored at -80°C until all of samples were collected.

### 3.4. Method of Pro-Oxidant Anti-Oxidant Balance Assay (PAB Assay) Calculation

The PAB assay is the only available test that can measure the balance of oxidants and antioxidants simultaneously in one experiment. It uses two different kinds of reactions: one is an enzymatic reaction where the chromogen TMB is oxidized to a color cation by peroxides and the second is a chemical reaction where the TMB cation is reduced to a colorless compound by antioxidants (1, 9). The photometric absorbance is then compared with the absorbance given by a series of standard solutions that are made by mixing varying proportions (0% -100%) of hydrogen peroxide with uric acid (9).

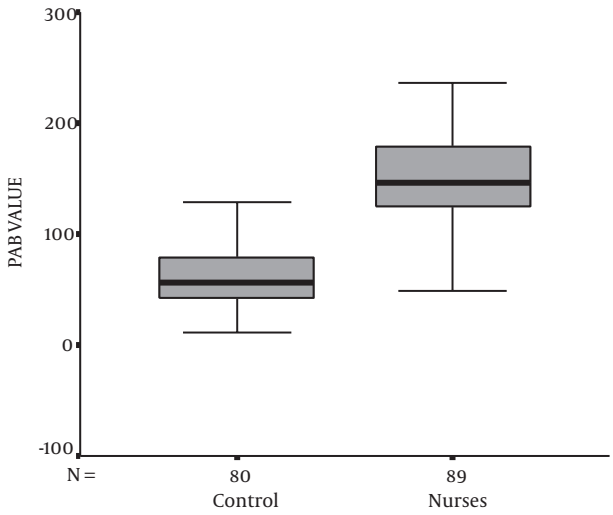
### 3.5. Statistics

We used independent-T test to compare HK index in two groups of nurses and control group. We used version 11 of SPSS for statistical analysis. Also we were classified nurses into two sex groups, males and females. We used also T- test to show relationship between genders in nurses with PAB values.

## 4. Results

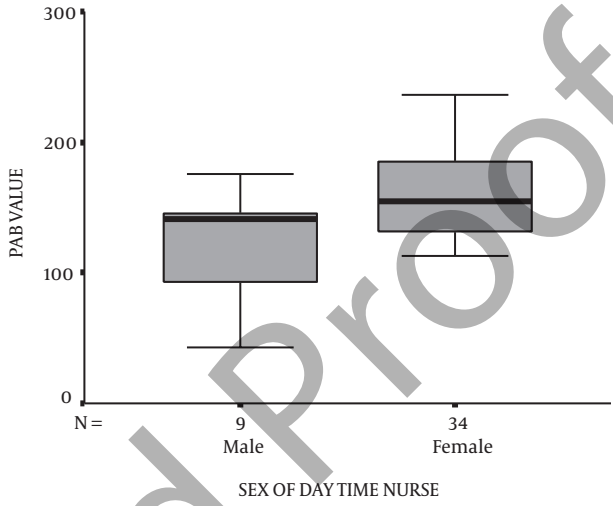
The PAB value of control group was 63/64 $\pm$ 34/41 SDS HK and in nurses who work at hospital was 152/28 $\pm$ 43/64 HK respectively (Figure 1). The serum PAB value between control group and nurses who worked at hospital was significant (P value = 0.001). HK is an arbitrary unit used by inventors of PAB method (Hamidi and Koliakos) (9). In day time nurses, there was a significant difference of PAB values between males (119.09 $\pm$ 47.14 HK) and females (162.53 $\pm$ 37.02 HK) (P = 0.005) (Figure 2). Also, in rotating day-night shift nurses there was a significant difference of PAB value between males (129.89 $\pm$ 35.76 HK) and females (170.46 $\pm$ 44.47 HK) (P = 0.002) (Figure 3).There was no

**Figure 1.** Comparison of Pro-Oxidant-Antioxidant Balance (PAB) Value Between Nurses and Control Group (Mean and the Standard Error/Standard Deviation of PAB Value)




Significant difference of PAB value between control group and case groups (P = 0.001).

**Figure 2.** Comparison of Pro-Oxidant-Antioxidant Balance (PAB) Value Between Male and Female Day-Time Nurses (Mean and the Standard Error/Standard Deviation of PAB Value)




In day time nurses, there was a significant difference of PAB value between males and females (P = 0.005).

significant difference of serum PAB between rotating day-night shift nurses (151.10 ± 45.02 HK) and daytime working nurses (153.44 ± 42.66 HK). A significant relationship was observed between PAB value and sex in nurses. There was no significant correlation between age and PAB value in control groups.

## 5. Discussion

In this study, the PAB assay showed the increased pro-oxidant level in shift-working and daytime nurses in comparison to control group, but there was no significant difference in serum PAB between shift-working (151.10 ± 45.02 SDS HK) compared with daytime nurses (153.44 ± 42.66 SDS HK).

Nursing is particularly one of the most stressful occupations. Stress in nurses and healthcare workers in hospitals may result in depression, anxiety, increase of blood pressure, lack of job satisfaction, decrease in efficiency, isolation from patients and, sickness absence (13, 14). Stress Oxidative due to excessive reactive oxygen species (ROS) obtained as a result of the stress conditions (1). Also, shift-working impairs normal circadian rhythms. In experimental studies, it has been reported that circadian rhythm effect on expression and/or activity of oxidative and anti-oxidative enzymes. Cellular mRNA levels of anti-oxidative enzymes such as glutathione peroxidase, superoxide dismutase (cellular and mitochondrial fraction), catalase, nitric oxide synthase, and heme oxidase changes by the circadian rhythm (15).

However Ulas et al have reported that there was no significant difference of oxidative stress parameters between day time and shift working nurses (16).

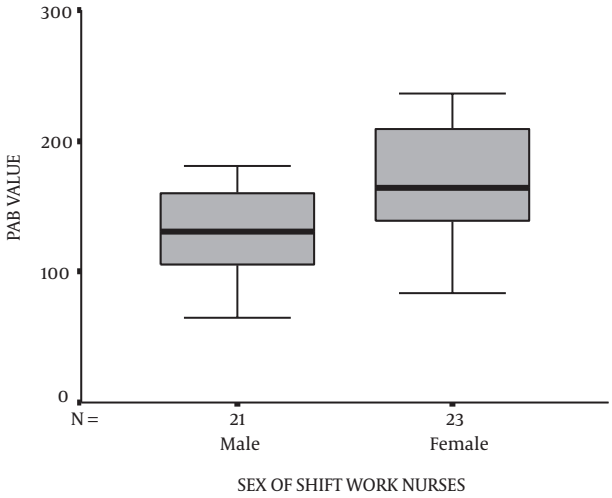
In a study looking at the relationship between severity of night shift work and antioxidant status in serum blood of nurses, markers of anti-oxidative processes and TBARS (lipid oxidation marker) did not differ between day time and shift working nurses (15).

Ishihara et al reported the increased marker of DNA oxidation, urinary 8-hydroxy deoxyguanosine (8-OH-dG) in nurses. Also, increased level of urinary 8-OH-dG was found in shift work in comparison to the result of those who were employed in part time. Their results showed a positive relationship between 8-OH-dG levels of nurses in the 35 - 45 age group and the individual scores of their depressive states (17).

In one study, MDA levels (lipid oxidation marker) has been compared in two group of nurses and control group, the result showed increase significantly in the nurses in comparison to the control group (1).

The other main secondary pathological mechanism in stressful challenges encompasses inflammatory response. Stressful conditions are related with changes in immune parameters, including increased natural immunity/inflammation. Chronic stressors have been correlated with suppression of both cellular and humoral measures

**Figure 3.** Comparison of Pro-Oxidant-Antioxidant Balance (PAB) Value Between Male and Female Shift- Working Nurses (Mean and the Standard Error/Standard Deviation of PAB Value)




In shift work nurses there was a significant difference of PAB value between males and females (P = 0.002).

and increasing the inflammatory factors (18).

Many different mechanisms can cause increasing cytokine production by oxidative stress. The increased pro-oxidant levels, acting similar second messengers, are well known to mediate inflammatory signaling and stimulate redox sensitive transcription factors such as signal transducer and activator of transcription (STAT), cAMP response element-binding protein (CREB), nuclear factor-$\kappa$-binding protein (NF- $\kappa$B), and activator protein1 (AP-1). The activation of these transcription factors lead to the transcriptional activation of inflammatory cytokines (tumor necrosis factor alpha (TNF$\alpha$), IL-1, IL-6, IL-8, and IL-18, etc.), chemokine (chemoattractant protein-1, etc.) and growth factors (transforming growth factor-$\beta$, monocyte, connective tissue growth factor, etc.) which could augment inflammatory complications via autocrine and paracrine pathways (19). In addition, it is displayed that antioxidants can down-regulate the pro-inflammatory cytokines through two possible mechanisms; firstly, through their effect on transcription factors that are regulated by redox status, and secondly by influencing prostaglandin E2 (PGE2) synthesis, which plays a key role in Th1 response and regulation of pro-inflammatory cytokines (20).

### 5.1. Conclusion

Our study confirmed that there was increased of serum PAB in nurses with day time and shift working job in comparison to control group (Figure 1), but we did not observed difference in PAB value in nurses with day time and nurses with shift working. Females of nurses have higher serum PAB concentration than males of nurses (Figures 2 and 3). Major problem with this study was getting samples after a period of time off meal. In further research, this easy explanation of oxidative stress in nurses can be practical to develop the effective antioxidant therapies for devising strategies to lessen the adverse effects of OS in this group.

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