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EXCESSIVE DAYTIME SLEEPINESS (EDS) AND CONCENTRATION OF ALKALINE PHOSPHATASE IN HUMAN SALIVA

R. K. Pradhan, Abhijit Ray and Chaynika Nag

School of Life Sciences, Pt. Ravishankar Shukla University, Raipur – 492 010, India.
e-mail: rkp299@gmail.com

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ABSTRACT : Adequate amount of sleep is essential for healthy living. Impairment of sleep has been reported to be associated with several health complication and increased sleepiness during daytime. Cigarette smoking and alcohol consumption is known to contribute reduction in sleep duration, deterioration of sleep quality and increased alkaline phosphatase activity. Although, estimation of this enzyme activity is carried out for diagnosis of other diseases, reports on increase of the level of enzyme activity with respect to sleepiness is lacking. In the present study, salivary alkaline phosphatase activity was measured on 61 individuals from four different groups i.e. control (19), smoker (14), alcoholic (16) and smoker & alcoholic (12) subjects. The DGKC kit was used for estimation of enzyme activity. EDS of all subjects were calculated using Epworth Sleepiness Scale. The level of salivary alkaline phosphates enzyme activity in smoker, alcoholic and smokers with alcoholics is significantly higher compared to control group. Increased enzyme activity with highest excessive daytime sleepiness (EDS) in smokers & alcoholic suggest that the sleep quality of these people is severely impaired. Further, it was also noticed that the impact of smoking and alcohol consumption significantly increase alkaline phosphatase level in smokers and alcoholic ($p<0.01$). Statistical analysis of data with SPSS10 detected a heightened association between level of enzyme activity and intensity of sleepiness ($p<0.01$). A significant association between enzyme activity and the level of sleepiness (ESS) suggests that measurement of alkaline phosphatase activity in saliva may be a good parameter as an indicator of sleepiness.

Keywords : Sleep Deprivation, ESS, EDS, Alkaline Phosphatase, smoking/alcoholic.

INTRODUCTION

Chronic deficiency in any biological requirements turned out as disease. This basic principle has been well established for nutrition, hydration, and oxygen. It also is believed to be true for sleep. About 7-8 hours sleep is considered optimum for healthy and productive life with great variation in requirement across human population. Smoking has been reported as one of the instrumental factors for sleep impairment (Bixler *et al,* 2005; Lemon, 1993) by contributing increase in obstructive sleep apnea (Kashyap *et al,* 2001; Zhang *et al,* 2006), reduction in sleep duration (Macgregor and Balding, 1988; Townsend *et al,* 1991), increase in sleep fragmentation, deterioration of sleep quality (Lesley *et al*, 1998; Stepanski *et al*, 1984) and increase in daytime sleepiness (Zielin Â *et al,* 1999). Similarly, excessive alcoholism also causes sleep disturbances (Lemon, 1993). Sleep disturbances associated with alcoholism include increased time required to fall asleep, frequent awakenings, and a decrease in subjective sleep quality associated with daytime fatigue (Aldrich, 1998). Smoking and alcohol consumption have been found to raise the level of alkaline phosphatase activity in serum (Webber et al. 2010; Padmavathi et al. 2009).

Reduction in optimum amount of sleep is accumulated as sleep debt and under chronic sleep deprivation leads to excessive daytime sleepiness (EDS) (Nag Chaynika and Pradhan R K, 2011). Although sleep deprivation is considered a risk factor for decreased academic performance, decreased cognitive function (Rodrigues *et al*, 2002), reduced immune response, (Vgontzas *et al*, 1998; Dinges *et al*, 1994) alter appetite and metabolic functions, (Knutson *et al*, 2007; Spiege 1999) and other health complications like diabetes, breast cancer, cardiovascular disease, and depressive disorder (Knutson *et al*, 2007; Janson, 1996; Asplund, 1996; Bixler, 2005; Knutson *et al*., 2007; Black *et al*, 2007; Empana, 2009), specific health impairments have not been definitively linked to sleep deprivation per se, and accordingly it has no descriptive clinical signs (Carol *et al*, 2000). However, despite the fact that deficiencies of this basic biological requirements can have life-threatening implications, a biochemical variables as a descriptive clinical signs for sleep deprivation in human is not well elucidated (Carol *et al*, 2009).

Rodents are champion sleepers, and the physiological correlates of sleep stages and wakefulness are comparable between rodents and humans (Zepelin, 1994). A progressive increase in the circulating concentration of alkaline phosphates has been reported in sleep deprived

rat without any histopathological evidence of liver damage (Everson, 1993, Everson and Wehr, 1991, and Gilliland *et. al,* 1984). We hypothesized that measurement of alkaline phosphatase concentration may be a potent marker of sleep deprivation. The present study is an attempt to investigate the relation between salivary alkaline phosphatase enzyme concentration and intensity of sleepiness.

MATERIAL AND METHOD

The present study was conducted on 61 individuals form Raipur district of Chhattisgarh state. The aim and objective of the study was explained and consent of each individual was obtained for collecting saliva sample. Persons suffering from any chronicle diseases or having other medical complication were excluded from the study. Saliva samples were collected from four different groups (1) persons who were neither smokers nor consume alcohol or any other sedatives or drugs (CONTROL), (2) persons who use to smoke more than 10 cigarettes per day (SMOKER), (3) persons who were habituated to consume 250-300 ml or more alcohol daily were included in the alcoholic category (ALCOHOLIC) and (4) persons who were smokers and habituated to consume alcohols (SMOKER ALCOHOLIC). Saliva samples were collected in morning hours between 8am to 9am to avoid time factor. Before collecting saliva, subjects were asked to wash their mouth with plain water and saliva was collected after 10 minutes. Each sample was collected in separate sterilized 1 ml eppendorf vials. Name of subject, categories and time of collection were marked on each vial. Collected samples of each group were further divided into two groups on the basis of their age. Persons below 35 years were considered as young and persons above 35 years were considered as old.

Alkaline phosphatase activity : DGKC kit was used for the measurement of alkaline phosphatase activity in saliva. 1000µl of reagent 1 was added to 20 µl saliva. The content was thoroughly mixed and incubated for 1 minute. Then reagent 2 was added and again mixed thoroughly. After 1 minute absorbance was recorded in spectrophotometer with a spectral light path of 1.5 cm three times at 1 minute intervals.

EDS : Excessive daytime sleepiness (EDS) of control, smoker, alcoholic, and smoker & alcoholic was measured by administering ESS instrument (Epworth sleepiness scale). The scale consists of eight items referred to chance of dozing in different situations. The questions are of multiple choice types and theoretical range of score is from 0-24. Score higher than 10 indicate presence of EDS. Data were analyzed with the help of CoStat (CoHort Software; Version: 4.02, ©1990) and SPSS 10. Other conventional statistical methods wherever required were also used.

RESULT

Alkaline phosphatase activity

Activity of alkaline phosphatase among different groups has been shown in Figure 1. The mean alkaline phosphatase activity were found to be (21.24±1.71) in control, (29.53±4.18) in smokers, (31.19±2.69) in alcoholic and (51.13±9.01) among the subjects having both alcohol and smoking habit (Figure 1). The concentratation of salivary alkaline phosphatase was significantly higher in smoker, alcohol and smoker alcoholic. When activities of alkaline phosphatase were compared among all four groups, a significant difference was observed except between smokers and alcoholic with highest activity among smokers alcoholic ($p<0.01$). When activity of alkaline phosphatase were compared between young and old age group, the level of alkaline phosphatase was observed to be significantly higher only in the old age group of smokers and alcoholic subjects ($p< 0.01$) (Table 1).

Excessive Daytime Sleepiness (EDS)

The mean level of sleepiness, obtained through self reported questionnaire, among control, smoker, alcoholic and smoker alcoholic group was found to be (7.00 ± 0.55), (9.42 ± 0.73), (10.38 ± 0.64), and (11.92 ± 0.63) respectively (Fig. 1). Result shows a significant difference ($p<0.01$) in average sleepiness score among different groups. Frequency of day time sleepiness in all the groups has been illustrated in figure in Fig. 2. Percentage of EDS among control, smokers, alcoholic and smoker alcoholic was found to be 21.05%, 50%, 56.25% and 100% respectively (Fig 2. Increase in day time sleepiness due to smoking and alcohol consumption was found to be statistically significant $p < 0.01$). Results indicate that 100 % people habituated to smoke and consume alcohol, suffer from EDS. Further a strong association between enzyme activity and intensity of sleepiness was also noticed. Stastical analysis of data revealed that alkaline phosphatase activity level is elevated with increase in the intensity of sleepiness ($p <0.01$) (Figure 3).

DISCUSSION

The global human population is changing into a 24/7 society with shortened sleep duration. Consequently, sleep disruption is profound in general population leading to EDS which in turn effects in behavioral, metabolic and endocrine responses (Naitoh, 1976). Detection of average 7 sleepiness score in control subjects is a clear indication of sleep deprivation in general population of urban area. The ESS score 6 or less than 6 is considered normal

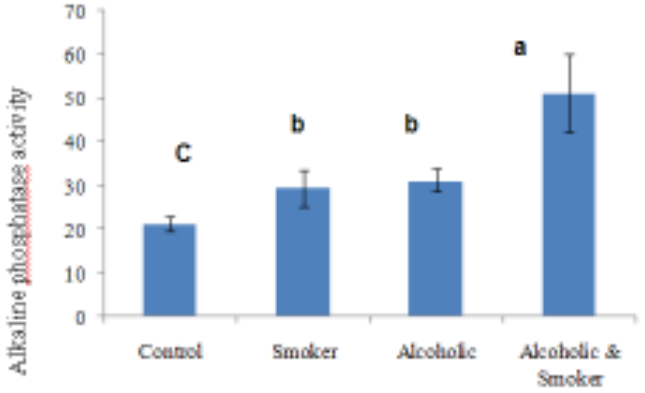


Fig. 1 : Comparison of alkaline phosphatase activity among different groups

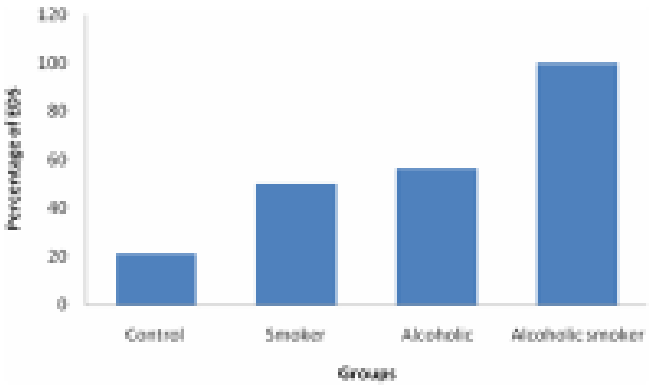


Fig. 2 : Prevalence of excessive daytime sleepiness.

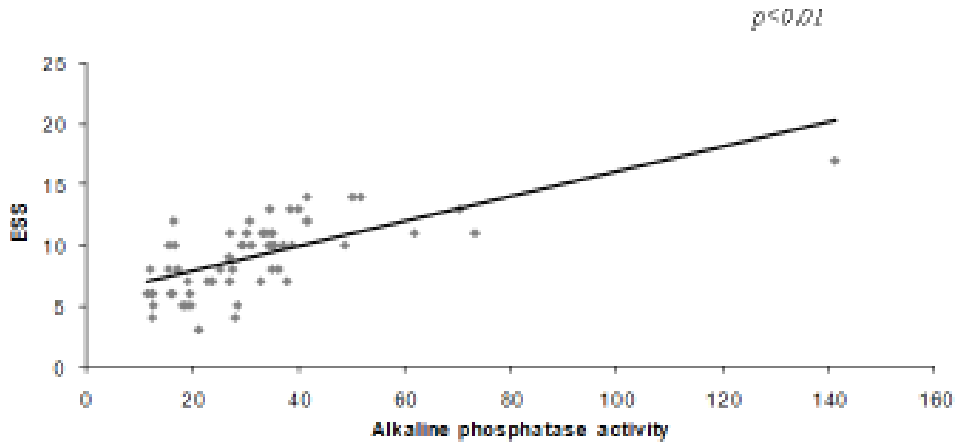


Fig. 3 : Intensity of excessive daytime sleepiness increases alkaline phosphatase activity.

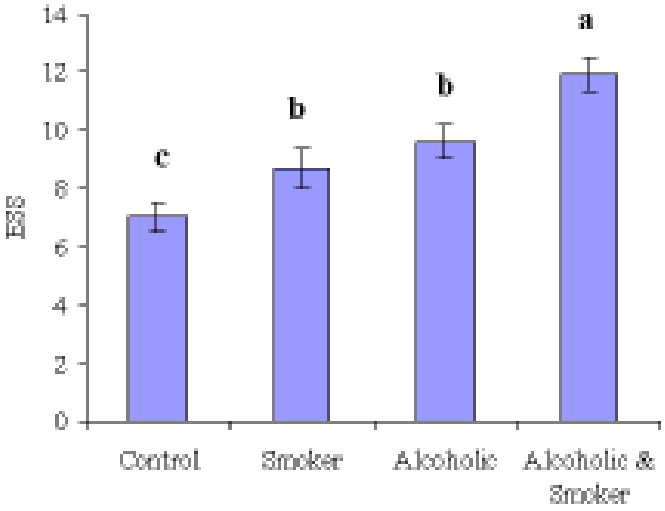


Fig. 4 : Average Epworth Sleepiness.

Table 1 : Average activity of alkaline phosphatase in different age groups

Factor	Young (Mean ± SE)	Old (Mean ± SE)	*t*- test
Control	21.54	20.82	*ns*
Smoker	19.37	37.14	$p< 0.01$
Alcoholic	25.34	37.03	$p< 0.01$
Smoker+ Alcoholic	45.43	53.98	*ns*

sleepiness level (Johns, 1991). The average sleepiness score in control, smoker, alcoholic and smoker alcoholic was found to be (7.00 ± 0.55), (9.42 ± 0.73), (10.38 ± 0.64), and (11.92 ± 0.63) respectively. The percentage of people suffering from EDS was detected as 21.05%, 50%, 56.25%, and 100% in control, smoker, alcoholic and smoker alcoholic subjects. Result shows that smoking and alcohols consumption intensifying increase of sleep deterioration in sleep deprived urban population. Further, it was also noticed that peoples who are smoker alcoholic invariably suffer from excessive daytime sleepiness. 100 % occurrence of EDS in smoker alcoholic may be attributed to synergetic effects of smoking and alcohols. Further, statistical analysis of data revealed that level of alkaline phosphatase activity is significantly elevated with increase in the intensity of sleepiness. This linear positive correlation suggests the qualification of the enzymes to be a good indicator of sleep deprivation in absence of any pathological sign. Rodents are champion sleepers, and the physiological correlates of sleep stages and wakefulness are comparable between rodents and humans (Zepelin, 1994). A progressive increase in the circulating concentration of alkaline phosphates has been reported in sleep deprived rat without any histopathological evidence of liver damage (Everson, 1993, Everson and Wehr, 1991, and Gilliland et. al. 1984).

In the present study, the estimated activity of alkaline phosphatase was detected as (21.24±1.71) in control, (29.53±4.18) in smokers, (31.19±2.69) in alcoholic and (51.13±9.01) among smoker alcoholic. The concentratation of salivary alkaline phosphatase was significantly higher in smoker, alcoholic and smoker alcoholic compared to control subjects. A remarkable increase in the enzyme activity can safely be ascribed to smoking and alcoholism (Webber et al. 2010; Padmavathi et al. 2009). When activities of alkaline phosphatase were compared among all four groups, a significant difference was observed except between smokers and alcoholic suggesting a equal potency of smoking and alcohols for raising alkaline phosphatase concentration. The impact of smoking and alcohol consumption on alkaline phosphatase seems to be more pronounced in older peoples. Comparison of alkaline phosphatase activity between young and old age group revealed that the level of alkaline phosphatase was significantly higher only in the old age group of smokers and alcoholic subjects.

Although, more study on larger sample size will be helpful to ascertain its applicability in clinical diagnosis, from the finding of the present study it can be concluded that (i) the impact of smoking and alcohol consumption on sleep may be more severe at latter age and (ii) measurement of alkaline phosphatase activity can be a good parameter for assessing sleepiness in absence of any histo-pathological evidence of liver damage.

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