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Editorial

Setting editorial goals ....

The IJPP has come a long way to its 56th year since its inception in 1958. It was started during the period when basic medical sciences in India were in their infancy. They needed support, conducive environment and a lot of efforts to nurture them. Our journal did serve this purpose to a great extent. It steered through the difficult times keeping its pace moving ahead. Throughout its journey the credibility of IJPP has been upheld by our valued authorship, contribution and enthusiastic readership. It has represented the face of Indian Physiology and Pharmacology. It has been the official journal of APPI and it has always given a very strong platform to a large number of scientists, teachers, physicians and our budding authors to express their scientific creativity and research.

At the very outset we, the Editorial Board, would like to set certain goal for ourselves. In this age of technology we should keep pace with the new trends and advancements. In near future we will achieve full online submission and tracking facility and full archival digitization for our journal. A journal does not fulfil its purpose if it is not available to its readers for their required needs in time. Thus, we wish to enlarge the volume and scope of the journal which is available at fast speed. We will improve our refereeing procedure by creating a referee resource for rigorous and constructive yet fast review procedure.

Our readers have always given us unconditional support and co-operation. A constant support, encouragement and timely feedback will certainly be necessary for us to keep up the system working with utmost efficiency. We commit ourselves for the advancement of scientific ethos and development of Physiology, Pharmacology and Allied Sciences.

Our roles and responsibilities as physiologists and pharmacologists have been changing. It reminds me the words of Prof B. K. Anand, who expressed them in editorial on the 40th year of publication of IJPP: “Now we have a transformed, dynamic society, with an accelerated pace of development, in almost all fields. .....we have to keep pace with that in our country. It has rightly been said in the words of ancient wisdom, that of all the marvels and mysteries of this universe, nothing is more marvellous than the human being and its body. We Physiologists have the privilege of trying to measure man, and at least in a small way APPI and IJPP are expected to help in the same”.

I convey my sincere thanks to Prof. D. Ghosh and his team who have taken great pains to continue this journey with their able editorial acumen.

K. K. DEEPAK
Executive Editor, IJPP

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Editorial

MENTORING AND OUTREACH STRATEGY OF APPI AND IJPP

In the annual meeting of the Association of Physiologists and Pharmacologist of India – APPICON2011 – in December 2011 held at the All India Institute of Medical Sciences (AIIMS), New Delhi, there was a special session on 'Vision: APPI 2020' organized by Professor H.N. Mallick of the Department of Physiology, AIIMS. In this meeting, the importance of mentoring and networking for scientists and teachers was discussed. In the General Body meeting, a resolution was taken to the effect that APPI and IJPP may proactively create a forum of 'mentoring' young and aspiring scientists and teachers towards a research career and thus help to facilitate the development of human resources in physiological and pharmacological sciences in India.

A mentor is a trusted guide and a counselor. A mentor can help in letting the mentee understand and practice what is needed for success in given field at a particular place and time. The umbra and penumbra of this success include individual career success to institutional development and over all scientific progress, respectively. Also, effective mentoring not only gives guaranteed outreach to the dependent, it also gives new kind of inputs to the provider as well. Thus, it bears the potentiality of 'win-win' story, if steered appropriately. As it has been observed in a workshop, '...although establishing a mentor-mentee relationship may take some time, the effort is not onerous and should be rewarding to both parties' (1).

The task however is not easy. The first hurdle is to identify an agency which can proactively take the charge. In our case, APPI and IJPP can take up the challenge. The second hard task is to identify a group of suitable mentors from different parts of India. A mentor is suitable when he or she can draw necessary output from the mentee, has right sense of responsibility, ethical values, discreteness, commitment and honesty. If necessary, program for training mentors is to be initiated under the auspices of APPI and IJPP to get it going effectively (2). It is also a huge task to make eco-systems of the mentee conducive to mentor-mentee interactions. 'Overall, the 'take-home message' is that a mentor should equip the mentee with necessary advice and tools to establish them as a researcher, while the mentee must be prepared to translate advice into action' (1).

The bottom line that scientists cannot 'go it alone' and that interacting with others can greatly benefit both mentor and mentee has be to appreciated and practiced in today's science. APPI and IJPP should play the role of catalysts in this strategy and be part of a silent scientific revolution in India.

DR. D. GHOSH
(Ex-Executive Editor)

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ROLE OF VENTROMEDIAL HYPOTHALAMUS ON ENERGY HOMEOSTASIS IN ALBINO RATS: EFFECT OF GENDER

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Abstract : Various brain areas like the ventromedial hypothalamus (VMH) are known to influence food intake and body weight. Though obesity is more common in females, the reports on gender difference in the neural regulation of energy homeostasis are not adequate. Therefore, the present study was conducted to assess the gender difference in the effect of VMH lesion on food intake (FI), body weight (BW), serum lipid profile, thyroid profile, glucose and insulin levels and glucose-insulin ratio (GIR) in Wistar albino rats. Twenty-four Wistar albino rats were divided equally into control and experimental groups with 6 male and 6 female rats in each. In the experimental group, bilateral electrolytic lesion of VMH was performed by stereotaxy and post-lesion parameters were recorded. In the control group, VMH sham lesion was made. Male-female difference in each parameter was determined. Following VMH lesion, FI was increased (females, $P<0.01$) and BW (males, $P<0.05$) and GIR decreased in males ($P<0.001$), which was significantly correlated with BW. T3 was more significantly correlated with FI and BW in females ($P<0.000$ and $P<0.001$). Following VMH lesion, male rats exhibited significant weight gain in the absence of proportionate hyperphagia indicating that weight-gain was mainly metabolic in nature. Also, the male rats developed more susceptibility to insulin resistance. The female rats developed resistance to weight-gain inspite of hyperphagia, which could be due to the higher T3 level.

Key words : ventromedial hypothalamus, food intake, body weight, lipid profile, thyroid profile, glucose-insulin ratio, Wistar rats

INTRODUCTION

Current WHO estimate suggests that over 1 billion people are overweight and over 300 million people are obese (1). Weight gain is due to an imbalance between energy expenditure and dietary intake (2). Regulation of body weight and adiposity is influenced by various factors such as nutritional, hereditary, social and environmental and so

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on (3). There are individuals who deposit fat even though they do not eat much and there are individuals who do not deposit fat even though they eat so much. This difference in adiposity could be due to the individual variation in regulation of body weight by central nervous system (4). However, our knowledge and understanding of neural regulation of adiposity is far from complete.

Hypothalamic areas like lateral hypothalamus (LH), ventromedial hypothalamus (VMH), arcuate nucleus (AR) and extrahypothalamic areas like nucleus septal medialis (NSM) and ventral medulla (VM) are known to influence food intake and body weight (4, 5). Nuclei within the hypothalamus integrate peripheral signals such as adiposity and calorie intake to regulate important pathways within the central nervous system controlling food intake and energy expenditure. Firmly established pathways involving orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) (NPY/AgRP); and the anorexigenic pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART), (POMC/CART) neurons project from the AR to other important hypothalamic nuclei, including the paraventricular (PVN), dorsomedial (DMN), VMH and LH nuclei (2). In addition there are many projections to and from the brainstem, cortical areas and reward pathways, which modulate food intake. Central circuits in the brain rely on peripheral signals indicating satiety levels and energy stores, as well as higher cortical factors such as emotional and reward pathways. The hypothalamus is subdivided into interconnecting nuclei, including the arcuate nucleus (AR), paraventricular nucleus (PVN), ventromedial nucleus (VMH), dorsomedial nucleus (DMN) and lateral hypothalamic area (LH). Neuronal pathways between these nuclei are organised into a complex network in which orexigenic and anorexigenic circuits influence food intake and energy expenditure (2).

Sympathetic activity is decreased in rats with ventromedial hypothalamic obesity (6). Leptin produced in white adipose tissue activates sympathetic nerve activity via the VMH (7). Hyperleptinemia is associated with increased body fat, body mass index, waist-hip ratio and insulin resistance (8). Free tri-iodothyronine is positively associated with insulin secretion and hyper-thyrotropinemia is relatively common in obese children (9). A difference exists in the regulation of feeding behaviour between males and females (10). Also, plasma leptin level differs in male and female subjects (11). Obesity is associated with various disorders like hypertension, diabetes, hyperlipidemia and hypothyroidism. However, the exact mechanisms that cause obesity are not known. Whether VMH directly influence the feeding behaviour or they alter the sympathetic activity and thereby alter energy intake and expenditure, is not clear.

Therefore, in the present study, we propose to assess the effect of gender on VMH regulation of food intake and adiposity in rats correlating with their lipid profile, thyroid profile and insulin resistance.

MATERIALS AND METHODS

Animals

After obtaining approval of the research council and animal ethics committee of

JIPMER, a total of 24 (12 males and 12 females) institute-bred healthy adult albino rats of Wistar strain weighing between 150-275 g were obtained for the study. Animals were randomly divided into two groups: Control group (sham-lesion group) and Experimental group (VMH-lesioned animals). The sample size in each group was 12 (6 male and 6 female rats). The rats were housed in individual plastic cages with wire lids. 12 hour light-dark cycle was maintained. Standard rodent chow and fresh tap water was available *ad libitum*. Rats were allowed to habituate in individual cages for 10 days before basal measurements were taken.

Basal food intake and body weight recordings

After 10 days of habituation, standard rodent chow and fresh tap water *ad libitum* was provided every day. Daily food intake and body weight was measured for one week to determine the mean 24-hour basal recordings.

Procedures

Anesthesia

Because the depth of anesthesia required for different procedures was different, the anesthetic agent used was different for different procedures. As light anesthesia was required for blood collection, ether was used as anesthetic agent. For lesion making, Inj. Ketamine (0.25 ml/250 gm body weight) was injected intraperitoneally. For sacrificing the animal, double the dose of ketamine was used.

Blood collection

Jugular venous puncture : From control rats approximately 1.5- 2 ml of blood was collected by this method for obtaining basal biochemical values after 7 days of basal recordings of BW and FI.

Cardiac puncture : Approximately 5 ml of blood was collected with the help of a syringe and needle by puncturing the left ventricle during sacrifice before fixation of brain.

Electrolytic nuclear lesion

For making lesion, the stereotaxic procedure was performed as described earlier (12). Bilateral electrolytic lesions of VMH were made by introducing electrodes into the nuclei on both sides and allowing the anodal current of 0.5 mA to pass through the electrode. In animals undergoing sham lesions, all the above-mentioned steps were followed except that no current was passed.

Parameters

The parameters recorded were :

Physical parameters

i) Body weight (g)

ii) Food intake (g)

Biochemical parameters (Fasting)

i) Serum Glucose (mg/dl)

ii) Serum Insulin (ng/ml)

iii) Glucose-insulin ratio (insulin resistance was calculated by obtaining the glucose-insulin ratio) (13).

iv) Serum Total Cholesterol (mg/dl)

v) Serum Triglycerides (mg/dl)

vi) Serum TSH (µlU/ml)

vii) Serum Total T3 (ng/dl)

viii) Serum Total T4 (ng/dl)

All these biochemical parameters were estimated following the standard procedures as practiced in the clinical laboratory of department of Biochemistry of JIPMER, Pondicherry.

Recording of parameters

Body weight (BW) : It was measured everyday with an electronic weighing machine for the entire period of the study.

Food intake (FI) : Food intake was measured daily. After blood collection and lesion/sham lesion procedure the animals were allowed to recover from the stress of the intervention for a period of seven days during which food intake and body weight was not measured.

Biochemical parameters

Collected blood was allowed to clot and then centrifuged to separate the serum. 0.5 ml of serum was used for analysis of fasting serum glucose and serum lipids (serum total cholesterol and serum triglycerides). The remaining serum samples were stored at –80°C in labelled containers for subsequent analyses of the following parameters :

Serum Insulin (Rat/mouse Insulin ELISA Kit, Millipore™, USA)

Serum TSH (Rat TSH ELISA Kit, Cusabio™, USA)

Serum Total T3 (Human TT3 RIA Kit, Immunotech ™, Czech)

Serum Total T4 (Human TT4 RIA Kit, Immunotech ™, Czech)

Insulin resistance was calculated by obtaining the glucose-insulin ratio (13).

Sacrifice

Animals were sacrificed following the standard procedure as described earlier (12).

Statistical analysis of data

For data analysis all values were expressed as mean±SD. Differences between means were compared by Student's *t* test. The differences among means were evaluated by one-way ANOVA (analysis of variance) using Graphpad InStat (Version 3, USA) software. Post hoc test was performed by Tukey Krammer multiple comparison test. The difference was considered statistically significant if probability of chance was less than 0.05 ($P<0.05$). For determining the correlation between the parameters Pearson's correlation test was used.

RESULTS

Control group

After undergoing sham lesions (Table I), the control male rats (n=6) did not show any significant difference in food intake (FI) and body weight (BW) from the pre- sham values. Similarly, biochemical parameters like serum glucose (SG), serum insulin (SI), serum total cholesterol (TC), serum triglycerides (TG), serum TSH, serum T3 (ST3) and serum T4 (ST4) also did not show any significant change from the pre-sham levels. But the GI ratio was significantly higher after sham procedure ($P<0.0156$).

Sham lesion did not significantly affect the FI, BW and biochemical parameters of the female control rats (n=6). But in female rats also the Gl ratio was significantly higher in the post sham period ($P<0.0285$) (Table II).

TABLE I: Food intake (FI), body weight (BW) and serum biochemical parameters in control male (n=6) rats before (pre sham) and after (post sham) lesion.

Parameters	*Pre-sham*	*Post-sham*	*P values*
FI (g)	15.59±1.32	16.56±1.90	0.3286
BW (g)	247.50±10.64	250.333±10.84	0.6579
Glucose (mg/dl)	72.33±10.91	74.16±7.30	0.7395
Insulin (ng/ml)	0.548±0.024	0.498±0.186	0.5285
G/I Ratio	131.98±11.90	148.91±7.84	0.0156
TC (mg/dl)	54.83±6.85	55.66±6.77	0.1231
TG (mg/dl)	55.83±5.60	55.33±13.79	0.9443
TSH (µlU/ml)	9.75±5.98	11.19±5.31	0.6683
T_3 (ng/dl)	19.16±4.15	25.19±9.77	0.1742
T_4 (µg/dl)	4.90±0.36	4.66±1.36	0.6416

Data expressed as mean±SD; The P<0.05 was considered significant. Analysis of data was done by Student's paired t test. Sham means the lesion making needle electrode was introduced in to the brain but current was not passed.

TABLE II: Food intake (FI), body weight (BW) and serum biochemical parameters in control female (n=6) rats before (pre-sham) and after (post-sham) lesion.

Parameters	*Pre-sham*	*Post-sham*	*P values*
FI (g)	12.21±1.56	13.32±1.87	0.2903
BW (g)	163.50±6.22	164.66±4.50	0.2387
Glucose (mg/dl)	66.83±7.08	73.83±7.16	0.1292
Insulin (ng/ml)	0.876±0.353	0.835±0.282	0.8286
G/I Ratio	76.28±8.24	88.42±8.20	0.0285
TC (mg/dl)	51.83±3.97	52.16±3.97	0.8893
TG (mg/dl)	107.01±14.39	114.50±16.73	0.4253
TSH (µlU/ml)	7.88±3.98	8.63±4.22	0.7580
T_3 (ng/dl)	33.80±4.25	36.36±8.08	0.3110
T_4 (µg/dl)	5.63±1.54	4.45±0.88	0.0798

Data expressed as mean±SD; The P<0.05 considered significant. Analysis of data was done by Student's paired t test. Sham means the lesion making needle electrode was introduced in to the brain but current was not passed.

On analyzing the gender differences of the control group of rats (Table III), it was seen that the female rats were significantly lower in BW (P<0.001) and their FI was much lesser than the male rats (P<0.05). In spite of that, there was no significant gender difference in SG and SI. However, the females had a lower Gl ratio than the males (P<0.001). Similarly, TSH & T4 did not show any appreciable gender difference. However, the gender difference in T3 did not exist following sham lesion. When the lipid profile was analyzed, it was observed that though TG levels in the females were significantly higher (P<0.001) the TC levels did not show any difference.

Experimental group

Effect of the lesion

After the lesion (Table IV), FI increased in both the sexes, but it was significant in females (P<0.01). Both males and females showed an increase in BW, but the increase was significant in males (P<0.05). The males showed a significant decrease (P<0.001) in Gl ratio whereas the females showed a significant increase (P<0.001) in Gl ratio. In the female rats, VMH lesion did not cause any significant change in any of the other parameters. In the male rats, there was significant increase in the SI levels (P<0.001) and a significant decrease in SG levels (P<0.01). Following VMH lesion, male rats also did not show any change in the lipid profile, however, there was a significant decrease in TSH levels (P<0.01) with no detectable change in ST3 and ST4.

Effect of gender

Even though the BW remained significantly lower (P<0.001) in female rats the difference in FI was not seen after VMH lesion. Post lesion, SG was significantly lower (P<0.01) with significantly higher SI levels (P<0.01) in male rats compared to females. Even though in control female

TABLE III : Food intake (FI), body weight (BW) and serum biochemical parameters in control male (n=6) and female (n=6) rats before (Pre sham) and after (post sham) lesion.

Parameters	*Pre-sham*		*Post-sham*		*P values*
	Male	*Female*	*Male*	*Female*	
FI (g)	15.59±1.32	12.21±1.56*	16.56±1.90$^{\#\#}$	13.32±1.87^{f}	0.0008
BW (g)	247.50±10.64	163.5±6.22***	250.33±10.84$^{\#\#\#}$	164.66±4.50***,^{fff}	<0.0001
Glucose (mg/dl)	72.33±10.91	66.83±7.08	74.16±7.302	73.83±7.16	0.4077
Insulin (ng/ml)	0.548±0.024	0.876±0.353	0.498±0.186	0.835±0.282	0.5480
G/I Ratio	131.98±11.40	76.28±8.52***	148.91±7.84*,$^{\#\#\#}$	88.42±8.20**,^{fff}	<0.0001
TC (mg/dl)	54.83±6.85	51.83±3.97	55.66±6.77	52.16±3.97	0.5608
TG (mg/dl)	55.83±5.60	107.01±14.39***	55.33±13.79$^{\#\#\#}$	114.50±16.73***,^{fff}	0.0001
TSH (µlU/ml)	9.75±4.98	7.88±3.98	11.19±5.31	8.63±4.22	0.6382
T_3 (ng/dl)	19.16±4.15	33.8±4.25**	25.19±9.77	36.36±8.083**	0.0013
T_4 (µg/dl)	4.90±0.36	5.63±1.54	4.66±1.36	4.45±0.88	0.3231

Data expressed in mean±SD. The * represents comparison with pre-sham males, $^{\#}$ represents comparison with pre-sham females; ^{f} represents comparison with post-sham male. The analysis of data was done by one-way ANOVA and post hoc by Tukey- Krammer test. *P<0.05; **P<0.01; ***P<0.001; $^{\#\#}$P<0.01; $^{\#\#\#}$P<0.001; ^{f}P<0.05; ^{fff}P<0.001.

TABLE IV : Comparison of food intake (FI), body weight (BW) and serum biochemical parameters in control and VMH lesion rats in both male (n=6) and female (n=6).

Parameters	*Control*		*Experimental*		*P values*
	Post-sham male	*Post-sham female*	*Post-lesion male*	*Post-lesion female*	
FI (g)	16.56±1.90	13.32±1.87*	19.16±2.11$^{\#\#\#}$	17.58±1.25$^{\#\#}$	0.0002
BW (g)	250.33±10.84	164.66±4.50***	268.66±13.20*,$^{\#\#\#}$	173.33±7.84***,^{fff}	0.0001
Glucose (mg/dl)	74.16±7.30	73.83±7.16	55.83±8.75**,$^{\#\#}$	74.33±10.32^{ff}	0.0022
Insulin (ng/ml)	0.498±0.186	0.835±0.282	1.32±0.42***,$^{\#}$	0.630±0.144^{ff}	0.003
G/I Ratio	148.91±7.84	88.42±8.20***	42.29±6.86***,$^{\#\#\#}$	117.98±11.56***,$^{\#\#\#,fff}$	<0.0001
TC (mg/dl)	55.66±6.77	52.16±3.97	52.01±2.96	55.50±6.25	0.4629
TG (mg/dl)	55.33±13.79	114.50±16.73***	53.16±14.56$^{\#\#\#}$	1 18.66±12.48***,^{fff}	0.0001
TSH (µlU/ml)	11.19±5.31	8.63±4.22	2.99±0.86**	5.65±1.75	0.0038
T_3 (ng/dl)	25.19±9.77	36.36±8.08	28.86±8.66	43.82±7.07**,^{f}	0.0053
T_4 (µg/dl)	4.66±1.36	4.45±0.883	5.04±0.98	4.86±0.761	0.7735

Data expressed in mean±SD. The * represents comparison with post sham males, $^{\#}$ represents comparison with post sham females; ^{f} represents comparison with post lesion male. The analysis of data was done by one-way ANOVA and post hoc by Tukey-Krammer test. *P<0.05; **P<0.01; ***P<0.001; $^{\#}$P<0.05; $^{\#\#}$P<0.01; $^{\#\#\#}$P<0.001; ^{f}P<0.05; ^{ff}P<0.01; ^{fff}P<0.001.

animals there was a significantly lower Gl ratio, after lesion the females had a significantly higher Gl ratio (P<0.001). Lipid profile did not have any appreciable change due to the lesion between the genders. Even though the thyroid profile did not show any gender difference in the control animals, after VMH lesion, female rats had significantly higher ST3 levels (P<0.05) (Table IV).

Correlation results

In the male and female rats with VMH lesion (Table VI), both FI and BW were significantly correlated with TSH and ST3. In both the genders, BW also showed a strong correlation with Gl ratio.

TABLE V : Correlation of change in food intake and body weight with alteration in total cholesterol (TC), glucose-insulin ratio (GIR), triglyceride (TG), thyroid stimulating hormone (TSH), tri-iodothyronine (T3) and thyroxine (T4) in male and female experimental rats (n=6, in each group) following lesion of VMH.

Parameters	*Food intake*				*Body weight*			
	Male		*Female*		*Male*		*Female*	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
GIR	–0.18	0.132	0.20	0.110	–0.50	0.010	–0.40	0.030
TC	–0.16	0.164	0.20	0.098	–0.17	0.150	0.22	0.086
TG	–0.15	0.186	0.18	0.146	–0.12	0.320	0.18	0.140
TSH	–0.30	0.034	–0.38	0.024	–0.36	0.048	–0.42	0.032
T3	0.50	0.014	0.75	0.001	0.48	0.003	0.70	0.000
T4	0.16	0.160	0.21	0.090	0.18	0.130	0.24	0.084

P<0.05 was considered significant.

DISCUSSION

Lesion of VMH in experimental animals resulted in increase in food intake in both males and females compared to the values of rats of their own control group (Table IV). This confirms the inhibitory nature of VMH on food intake, for which VMH has been designated as satiety center (5). However, the increase in food intake in female was more significant (P<0.01) compared to the nonsignificant increase in males indicating that satiation effect induced by VMH in females is more than that of the males.

Though there was increase in body weight in both male and female rats compared to their own control rats following VMH lesion, the increase was significant in male rats (P<0.05) and not significant in female rats (Table IV). This indicated that body weight gain in male rats was more than the female rats. This is a very interesting finding that body weight gain in male was more than female inspite of food intake was more in females. This indicates the dissociation of mechanisms controlled by VMH regulating food intake and body weight. It is well known that lesion of VMH leads to massive obesity in four to six weeks, which is called as 'hypothalamic obesity' (14). In our study, the body weight gain was not massive, because we had recorded all the parameters in third week following lesion, when body weight gain was in the first phase (the rising phase) as we were interested to assess the acute effects of lesions in the present study.

Important parameters of energy homeostasis are levels of serum glucose and insulin. In the present study, serum glucose concentration was significantly decreased in male rats (P<0.01), but not in female rats compared to their control counterparts following VMH lesion (Table IV). There was no male-female difference in serum glucose levels in the control rats, but post-lesion the difference was highly significant due to acute hypoglycemia in males (P<0.01). The decrease in serum glucose in males could primarily be due to increased plasma insulin in these rats. Hyperinsulinemia is a common feature of lesion of VMH (6, 14, 15, 16). Though many studies report a state of euglycemia in the presence of sustained hyperinsulinemia and suggest insulin resistance even in the presence of normal serum glucose following VMH lesion (6, 15);

hypoglycemia was observed in the present study. The difference could be due to the timing of observation, as many investigators have assessed the parameters after one month of lesion by which pancreatic adaptation occurs to VMH-induced obesity (16). Therefore, we had assessed the acute effects of lesion in the present study. It has been reported that in the acute phase after VMH lesion, rats are hyperinsulinemic and hypersensitive to insulin; and in the later phase when obesity is well established, VMH lesion rats become insulin resistant (15).

The alteration in energy homeostasis in VMH-lesion is considered mainly due to the alteration in autonomic (vagosympathetic) output (6). It has been observed that VMH lesion results in reduced sympathetic and increased parasympathetic activity (6, 17, 18). It is known that sympathetic activation causes body weight loss and parasympathetic activation causes body weight gain. Therefore, sympathovagal balance is the major contributor to energy homeostasis of the body (19).

Moreover, the glucose-insulin ratio (GIR), an index of insulin resistance was significantly decreased in males ($P<0.001$) and increased in females ($P<0.001$) following VMH lesion. The decrease in GIR indicates insulin resistance and increase in GIR is associated with increased insulin sensitivity (13). Therefore, from the present study it appears that insulin resistance is induced by VMH lesion in male rats, whereas female rats are protected from this. This was further supported by the findings of the present study that the body weight but not the food intake was significantly correlated with the Gl Ratio in control rats (Table V). Moreover, in experimental rats, body weight gain (but not the increase in food intake) following VMH lesion was significantly correlated with Gl Ratio, in which the correlation was more pronounced for male rats compared to female rats (Table VI). All these findings indicate that male rats following VMH lesion are more susceptible to develop insulin resistance.

There was no difference in alteration in total cholesterol and triglycerides in experimental rats following VMH lesion compared to controls rats that had undergone sham lesion (Table III), which indicates that VMH has no direct influence on lipid profile in rats. Moreover, there was significant correlation of food intake and body weight with lipid profile parameters in rats before and after lesion.

In the present study, there was significant decrease in TSH level in rats following VMH lesion, in which decrease was more pronounced in male rats ($P<0.01$) compared to female rats (not significant) (Table IV). Also, T3 level was increased (though not significant) following VMH lesion. These findings indicate that VMH lesion induces some degree of hyperthyroidism (low TSH and high T3), which may contribute to alteration in energy homeostasis. The influence of VMH on thyroid hormone secretion is executed via hypothalamo-pituitary-thyroid axis of neurohumoral control. Though the increase in T3 in male and female rats was not significant compared to their control rats, there was a significantly high T3 in female rats compared to male rats ($P<0.05$). As thyroid hormones facilitate metabolism and decreased adiposity, a higher level of T3 in female rats

may be among the contributory factors for less body weights gain following VMH lesion in these animals. Increase in T3 level was significantly correlated with increase in body weight in experimental rats (Table V), indicating the direct association of thyroid hormones with food intake and body weight. The degree of correlation of both TSH and T3 was more in females compared to males, indicating thyroid control of food intake and body weight is more prominent in females.

From the present study, it is confirmed that neurophysiological control of energy homeostasis in rat model is mainly dictated by hypothalamic influences originating primarily from ventromedial hypothalamus. It appears that VMH controls energy balance by its influence through sympathovagal output, hypothalamopituitary-thyroid axis and hypothalamopituitary-pancreas axis.

Conclusion

To conclude VMH is the important center for satiety and adiposity in rat models and has differential influence on control of food intake and body weight in male and female rats. Lesion of VMH predisposes the male rats to insulin resistance, but not the female rats. Hypothalamo-pituitary-thyroid axis is involved in VMH control of energy homeostasis, which appears to be stronger in females than in males.

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IMPEDANCE CARDIOGRAPHY FOR MONITORING CHANGES IN CARDIAC OUTPUT

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Abstract : Impedance Cardiography (ICG) is a non invasive method useful for continuous monitoring of cardiac output but, it still has not found wide usage for measuring cardiac output in clinics and research. Most studies focused on comparing the cardiac output measured at rest with reference methods. In the present study we evaluated the validity of ICG against Doppler Echocardiography (DE) in measuring cardiac output changes that occur during static exercise. Cardiac output of 30 healthy males between 18-26 yrs of age was measured during supine rest, during and 5 min after completion of 3 minute static exercise by ICG and DE. The increase in cardiac output during exercise measured with ICG and DE does not differ significantly (1.04±0.72 L/min and 1.05±1.24 L/min respectively) and has significantly high correlation (r=0.76, P<0.001). The bias and limits of agreement are (–0.01±0.83) in acceptable limits. The pooled means of cardiac output measured by ICG and DE do not differ significantly and bears a significant correlation (r=0.812, P<0.001). The bias (d±s) calculated is 0.15±0.64 L/min. ICG could provide valid information regarding the relative changes in cardiac output.

Key words : cardiac output echocardiography monitoring stroke volume

INTRODUCTION

Impedance Plethysmography (IP), a noninvasive technique, originally was described for measurement of blood flow (1). Application of this technique, has been extended for determining cardiac output by recording changes in impedance that occur as blood is pumped into the aorta (2). Cardiac output and stroke volume represents the functional expression of cardiovascular performance and can be used to monitor changes in a patient's hemodynamic status. Impedance Cardiography (ICG) have been found potentially useful in monitoring cardiac output of patient in inpatient settings

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as the changes in impedance and thus stroke volume can be recorded and displayed continuously on screen. In critical care settings, monitoring of hemodynamic parameters by pulmonary artery catheterization though useful but have received several criticism because of higher risk of infections and increase mortality owing to the invasive nature of the technique (3, 4). Recently, Silver et. al. (2004) have pointed out that owing to high cost involved in pulmonary artery catheterization this procedure may be replaced by noninvasive ICG (5). There are reports suggesting ICG is fairly comparable to the dye dilution, thermodilution techniques and also to Doppler Echocardiography (DE), for measurement of cardiac output at rest (6,7,8). Results from studies comparing the absolute values of cardiac output at rest may not be extrapolated to situations where gauging the fluctuations in cardiac output rather than absolute values were needed for example in exercise physiology research and in intensive care setups.

In the present study we evaluated the validity of ICG against DE,. as a reference method for comparison, in measuring the changes in cardiac output that occurs during static exercise. DE has shown a good correlation with invasive methods for measurement of cardiac output and is the most accepted non invasive method for determining cardiac output (9, 10). DE is particularly advantageous to be used in this exercise paradigm as it is capable of determining the cardiac output quickly and easily (8, 11).

In the present study, hand grip isometric exercise paradigm has been employed to register changes in cardiac output/stroke volume in healthy subjects. The change in cardiac output, which takes place during isotonic exercise, has been measured with ICG and DE.

MATERIAL AND METHODS

Subjects

30 healthy males, between 18-26 yrs of age, were recruited for this study. Brief history and clinical examination was done to rule out cardiovascular and respiratory diseases. Informed consent was obtained after explaining the nature of study to the subjects and ethical clearance was taken from the institutional ethics committee. Subjects were called 2 hrs after light break fast in morning.

Devices

Doppler echocardiography

The diameter of the left ventricular outflow tract was used to calculate the cross sectional area, assuming a circular profile. Measurements were made by cross sectional echocardiography in the parasternal long axis view (Hewlett-Packard Sonos 5500® Echocardiography System). Stroke volume and cardiac output were measured using protocols as described earlier (12).

Impedance cardiography

The Impedance Plethysmograph (NICOMON, Larsen & Turbo India Ltd.) was connected to the subject via four pairs of button electrodes as described previously (13). The lower thoracic voltage sensing electrodes were placed at the level of the xiphisternum in the mid-axillary lines and

the cervical sensing electrodes were positioned laterally at the base of the neck as close as possible to the clavicles. The "current" electrodes, delivering an alternating current of 4 mA at 48 kHz, were placed with one pair 5 cm above the cervical sensing electrodes and the other pair 5 cm below the thoracic sensing electrodes. The recorded waveforms of changes in impedance are analyzed as a function of time using Kubicek's equation and area under the curve represents maximum change in blood flow during systole i.e. stroke volume. The NICOMON automatically averages stroke volume over ten cardiac cycles and displays the cardiac output using corresponding R-R intervals which are simultaneously recorded by it.

Protocol

Baseline cardiac output was measured in supine position after 30 min of rest by Impedance Cardiography. Simultaneously trained cardiac physician recorded cardiac output by echocardiography. The subject was asked to perform the static exercise for 3 minutes using hand grip dynamometer with sub-maximal static effort at 30% of maximum voluntary contraction. The cardiac output was again determined during and 5 minutes after completion of exercise.

Statistical analysis

Paired 't'-test was used to compare the pairs of cardiac output measured by Impedance Cardiography and Doppler Echocardiography measured three times i.e. at rest, during exercise and 5 min after exercise. The changes in cardiac output measured as both increase during exercise and decrease after exercise, were calculated and were compared using correlation analysis. As the correlation analysis may be misleading for assessing validity, agreement was also calculated using Bland & Altman plots (14). All three pairs of data were pooled and correlation analysis was done between cardiac output measured by ICG and DE and limits of agreement, P value less than 0.05 was taken as significant.

RESULTS

A total of 30 subjects who were evaluated were healthy from clinical point of view and their baseline characteristics are presented in Table I.

Cardiac outputs, measured before exercise during rest, during exercise and 5 minute of rest after exercise by Impedance Cardiography and Doppler Echocardiography, are presented in Table II. The mean cardiac

TABLE I: Baseline characteristics of the subjects. Data are expressed as Mean±SD.

No. of subjects (n)	30
Sex (M:F)	18:12
Age (in yrs)	21.5±2.4
Height (in cms)	171.1±5.3
Weight (in kgs)	61.2±7.5
BMI (kg/cm^2)	20.97±2.7
Systolic blood pressure (in mmHg)	120.9±6.5
Diastolic blood pressure (in mmHg)	75.2±5.9

TABLE II: Cardiac output obtained during rest, during exercise and 5 minute after rest as measured by Impedance Cardiography and Doppler Echocardiography. N=30, Values represents Mean±SD of cardiac output in L/MIN).

Cardiac Output	*At Rest*	*During Exercise*	*After Exercise*
Impedance cardiography	4.87±0.83	6.01±0.98	5.07±0.87
Doppler echocardiography	4.98±0.88	6.17±1.01	5.26±0.95

output values measured with two techniques do not differ significantly (P>0.05) either during rest, during exercise or after 5 minutes rest.

The pooled mean of cardiac output measured by Impedance Cardiography and Doppler echocardiography are 5.32±1.01 L/min. and 5.47±1.07 L/min respectively, which again do not differ significantly (P>0.1).

Correlation analysis revealed a significant correlation (r=0.812, P<0.001) between the pooled cardiac outputs measured with Impedance Cardiography and Doppler echocardiography (Scatter plot Shown in Fig. 1).

To test the validity of Impedance Cardiography for measuring cardiac output, mean difference (d) and standard deviation (s) was determined and limits of agreement were calculated as d + 1.96 s and d – 1.96 s (Table III). And for graphical representation of limits of agreement Bland & Airman graphs are presented (Fig. 2) The bias (expressed as d ± s) for cardiac output measurement by Impedance Cardiography and Doppler Echocardiography was 0.15±0.64 L/min.

TABLE III: Limits of agreement between cardiac output measured measured with Impedance Cardiography and Doppler Echocardiography. d=bias, s=standard deviation.

Cardiac output measured with ICG and DE	d	s	d+1.96s	d–1.96s
Pooled data before exercise, during exercise and rest after exercise	0.15	0.64	1.41	–1.10
Change in cardiac output during exercise and before exercise	–0.01	0.83	1.61	–1.64

The changes in cardiac output (Mean±SD) during and after exercise measured with Impedance Cardiography and Doppler echocardiography was 1.04±0.72 L/min and 1.05±1.24 L/min respectively which did not differ significantly (P>0.05). The detected

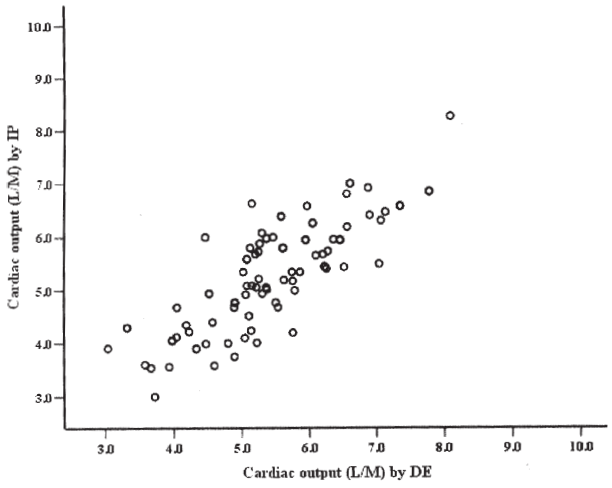

Fig. 1: Correlation between cardiac out measured by Doppler Echocardiography (DE) and Impedance Plethysmography (IP).

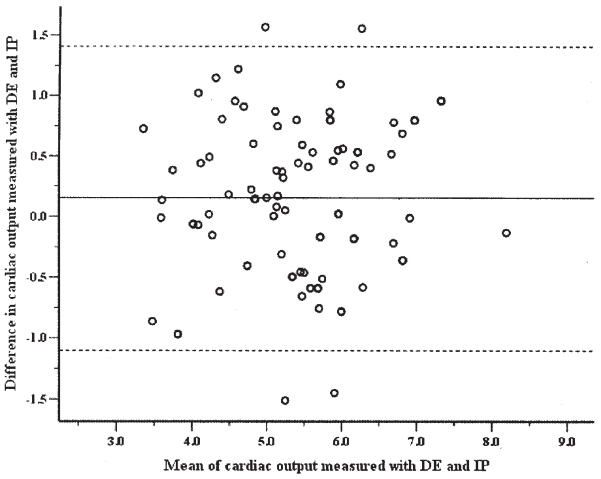

Fig. 3: Bland & Altman plot showing limits of agreement [solid reference line represents mean difference (d) and dotted reference lines represent d±1.96s].

change in cardiac output with two techniques have significantly high correlation (r=0.76, P<0.001). The bias and limits of agreement have been presented in Table III and graphical representation in Fig. 3.

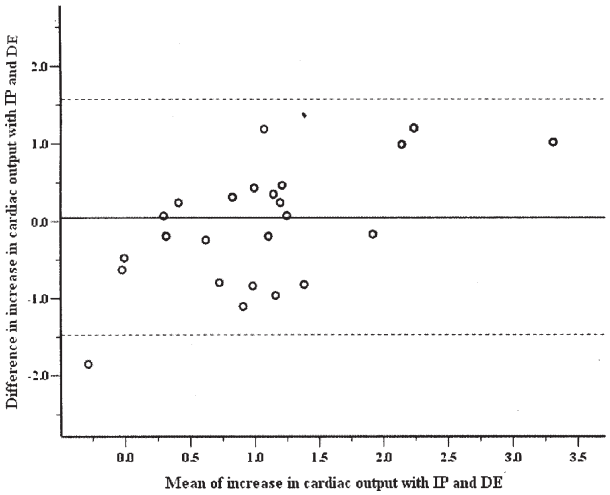

Fig. 3: Bland & Altman plot showing limits of agreement [solid reference line represents mean difference (d) and dotted reference lines represent d±1.96s].

DISCUSSION

After introduction of Impedance Cardiography for cardiac output in 1985 by Muji et. al., (2) the validity of this technique has been in question despite a large no. of published reports comparing it with Thermodilution and Doppler Echocardiographic methods (10, 15, 16). Most of the studies focused on comparing the absolute values of cardiac output at rest therefore their results may not be extrapolated to situations where gauging the fluctuations in cardiac output is important as in intensive care setups.

For cardiac output measurement, as Pick method is time consuming and invasive, DE is particularly advantageous in this exercise paradigm as it is quicker and easier (8, 11). In the present study we evaluated the validity of ICG using Doppler Echocardiography as a reference method for measuring the changes in cardiac output that occurs during static exercise.

The cardiac output by Impedance Cardiography did not differ from that by Doppler Echocardiography measured at rest, during static exercise and after static exercise. The pooled Cardiac outputs at rest, during and after static exercise also did not differ significantly measured by Impedance Cardiography and Doppler echocardiography. The correlation coefficient for the pooled data was also quite high signifying the fact that Impedance Cardiography is as good as Doppler echocardiography for measuring cardiac output over a wide range (2.8 L – 8.3 L). The high correlation levels are in accordance with the previous studies over a limited range of cardiac output measurement (10, 17, 18) Moreover, the limits of agreement calculated for pooled data is within acceptable limits and are in accordance with previous studies (17-20). Thus high correlation and acceptable limits of agreement signify the reliability of Impedance Cardiography in measuring cardiac output over wide range.

The reliability of Impedance Cardiography for gauging the changes in cardiac output has also been evaluated in this study and again the changes measured with Impedance Cardiography correlates well with the results with Doppler Echocardiography. The limits of agreement are favorable in this case too. Previous reports have established excellent

preciseness and reproducibility of Impedance Cardiography (15, 21) therefore; the Impedance Cardiography can be expected to become the standard modality for monitoring cardiac output in intensive care setups as well as exercise physiology research and similar applications.

Recently two multicentre studies PREDICT study (Prospective Evaluation and identification of Decompensation by Impedance Cardiography Test) and BIG study (Bioimpedance cardiography) have been published and show encouraging results for application of ICG in the management of heart failure patients (22, 23). In addition, the data from the critically ill patients with altered states of cardiac output needs to be explored to establish the use of Impedance Cardiography in critical care settings.

Doppler Echocardiography itself may not be used for monitoring purposes as it is operator dependent technique which needs expertise. On other hand ICG does not need expertise and is a cost effective method which may be used for continuous monitoring purpose.

In this investigation, the study population was limited to healthy young subjects and as the Impedance Cardiography depends on the pressure area relationship, age related changes in the arterial system e.g. arteriosclerosis, may influence the results Although the aortic compliance did not depend on the degree of sclerosis for pressure in the physiological range (24) the influence of increased arterial wall stiffness with aging would be prominent with increased blood flow and pressure during exercise. Furthermore, the influence of an enhanced sympathetic activity during intense exercise on the propagation of a pressure pulse might be increased in aging therefore, further investigation in various age groups are required. In addition, more data regarding its application in patients with different clinical conditions with altered states of cardiac output needs to be explored.

In conclusion summary, we tested the validity of non invasive cardiovascular parameters measured by Impedance Cardiography during sub maximal static exercise in healthy young males. The good agreement between Impedance Cardiography and Doppler Echocardiography both for absolute values and for gauging changes in cardiac output suggest that Impedance Cardiography could provide valid information regarding the relative changes in cardiac output during sub maximal static exercise in healthy young humans. This conclusion can be extrapolated to critical care setup where beat to measurement of cardiac output may provide crucial information regarding the hemodynamic status of the patient. More data from patients may be helpful in judging the clinical utility of the impedance Cardiography for continuous monitoring of cardiac output non-invasively.

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LEVEL OF NITRIC OXIDE AND ANTIOXIDANT VITAMINS IN SICKLE CELL ANAEMIA PATIENTS

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Abstract : Sickle cell anaemia (SCA) is characterized with sever anaemia and vasoocclussive episodes. Nitric Oxide (NO) a potential vasodilator, synthesized from various cells including endothelial cell. However SCA is associated with endothelial dysfunction, a measure cognitive factor for pulmonary hypertension (PH) and vasoocclussive crisis. The present study was attempted to evaluate level of serum NO and plasma antioxidant vitamins A, E and C in homozygous (n=30) and heterozygous (n=30) sickle cell patients and compared with age and sex matched healthy controls (n=30). We found, significantly (P<0.0001) elevated level of serum NO and significantly (P<0.0001) depleted antioxidant vitamins in homozygous and heterozygous sickle cell patients compared to healthy controls. Our study reveals that oxidative stress may be a responsible factor for the reduced bioavailability of NO which can impair the vasodilation in sickle cell patients.

Key words : sickle cell anaemia, vasoocclussive crisis, nitric oxide, oxidative stress, antioxidant vitamins

INTRODUCTION

Sickle cell anaemia (SCA) was the first disease to be characterized at the molecular level, but some of mechanisms underlying pathophysiology remain unexplained. Similar to the vascular disease like atherosclerosis, SCA is associated with chronic inflammation and ischemia-reperfusion injury due to the occlusion of rigid sickle erythrocytes in capillary beds (1, 2). Vasoocclusive consequences lead to acute episodic pain, infection, cerebral infarction, acute chest syndrome, splenic sequestration, end organ damage and early death (3).

Nitric Oxide (NO) synthesized in an oxygen dependant reaction catalyzed by nitric

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oxide synthase (NOS), converting L-arginine to citrulline. Nitric oxide synthase has 3 major isoforms; neuronal (nNOS) and endothelial (eNOS) being constitutive, and inducible (iNOS). iNOS is expressed after the induction by lipopolysaccharide (LPS), interleukins (IL-1, IL-11), and tumor necrosis factor (TNF-α) the inflammatory mediators in various inflammatory disorders such as inflammatory bowel disease, left ventricular failure, metastatic melanoma and neutropenic sepsis (4, 5).

Elevated level of malondialdehyde (MDA), a lipid peroxidation product and superoxide dismutase (SOD) activity has been also observed in previous studies suggesting excessive formation of reactive oxygen species (ROS) by sickle cells. Lowered level of antioxidant vitamins and enzyme activities were also reported in SCA patients (6, 7). These ROS can react with NO converting into more potent reactive NO species (RNOS) which may damage the cell membrane. This may exaggerate the sickling and hemolytic consequences in sickle cell anaemia. Further, hemolysis may derange the NO metabolism that reduces the bioavailability of NO leading to poor vasodilation and vasoocclussive process.

Therefore, measurement of NO along with antioxidant vitamins can provide a simple and potential inflammatory as well as oxidative stress marker in sickle cell anaemia.

MATERIAL AND METHODS

The present study was carried out in the Department of Biochemistry, Annasaheb Chudaman Patil Memorial Medical College and sickle cell centre, Shri Bhausaheb Hire Government Medical College, Dhule, Maharashtra. Prior to start the study, local ethical clearance was obtained. A total population of 90 subjects were enrolled in the study, including age and sex matched 30 (15 male and 15 female) healthy controls (HbAA), 30 (15 male and 15 female) homozygous (HbSS) and 30 (15 male and 15 female) heterozygous (HbAS) sickle cell patients on the basis of solubility test and HPLC analysis of blood. Subjects were excluded from the study using criteria of age <15 years, other than HbAS and HbSS pattern, past three month history of crisis, blood transfusion, treatment with hydroxyurea, use of vitamins and trace elements supplementation and pregnancy.

After obtaining the written consent from all the subjects included in the study a total of 7 ml of blood withdrawn aseptically from the antecubital vein. From this approximately 3 ml blood in EDTA (0.47 mol/L K3-EDTA) container and 4 ml blood in plain container drawn to obtain plasma and serum respectively. Samples were centrifuged at 3000 rpm for 10 min to separate the plasma and serum. For the estimation of nitric oxide, serum was deproteinised first and nitrate was reduced to nitrite by cadmium granule reduction method which then coupling with N-napthyethylenediamine to give pink coloured complex as per the method of Cortas and Wakid (8). Plasma Vitamin A measured by Carr-Price reaction in which blue colour complex was formed (9). Plasma Vitamin E determined by Baker and Frank method which is based on reduction of ferrous ions which forms a red coloured complex with α-α^1 dipyridyl (10). Plasma Vitamin C measured by Caraway method based on the reaction

with dinitrophenyl hydrazine (11).

Data were analyzed using SPSS program version 16.0. Values were expressed as means±SD, significance of the mean difference between SCA patient and control was assessed by statistical paired student T test. Pearson's correlation coefficient used for the correlation assessment.

RESULTS

Data of Table No. I shows, significantly (P<0.0001) increased level of serum NO and significantly (P<0.0001) decreased antioxidant vitamins (A, E and C) in heterozygous as well as homozygous sickle cell anaemia patients compared to controls. We also seen negative correlation between plasma NO level and Vitamin A (r= –0.62, P<0.01), Vitamin E (r=–0.72, P<0.01) and Vitamin C (r=–0.64, P<0.01) in homozygous sickle cell patients.

DISCUSSION

In the present study, we observed the elevated level of serum NO and depleted antioxidant vitamins in SCA patients compared to the control group. Acute painful vasoocclussive crisis (VOC) is one of the earliest manifestations of sickle cell disease (SCO) may occur in early age of life (3). Endothelial dysfunction is associated with intravascular hemolysis, reduced nitric oxide (NO) bioavailability, oxidative stress and inflammation. This leads to vasomotor instability and ultimately producing a proliferative vasculopathy, leading to the development of the pulmonary hypertension (PH) in adult age. Pulmonary hypertension, a common complication is a major predictor of the mortality rate (12, 13). Reduced bioavailability of NO, act as predisposing factor for the vasoocclusion by promoting RBC adhesion and impairing the regional regulation of blood flow (14).

Among endothelial mediators, nitric oxide (NO) regulates the normal vascular tone, cellular adhesion, platelet aggregation, and thrombosis. Various studies have demonstrated a state of resistance to the vasodilation due to eNOS mediated impaired blood flow (15, 16). Recently, it has been shown that, this state of NO resistance to the endogenous and exogenous NO, correlated with increased plasma hemoglobin levels which coupled to hemolytic rate and

TABLE I: Mean (SD) levels of serum NO and antioxidant vitamins in controls (HbAA), heterozygous (HbAS) and homozygous (HbSS) sickle cell patients.

Parameters	*Controls*	*Heterozygous*	*Homozygous*
N	30	30	30
Age in years	15–60	15–60	15–60
Male	15	15	15
Female	15	15	15
Nitric Oxide µ mol/l	32.11±6.49	63.41±16.75*	82.88±33.18*
Vitamin A µg/dl	42.73±4.88	30.76±2.44*	23.43±2.82*
Vitamin E µg/dl	82.29±3.06	56.97±4.47*	42.86±3.73*
Vitamin C µg/dl	85.05±3.59	70.26±5.21*	46.81±6.62*

*-P<0.0001 – Control Vs Homozygous Sickle cell anaemia.
*-P<0.0001 – Control Vs Heterozygous Sickle cell anaemia.

oxidant stress (13, 17). After, the hemolysis hemoglobin is decompartmentalized and released into plasma, where it rapidly reacts with NO and destroys it (12). This results in the abnormally increased consumption of NO forming NO free radicals and ultimately inhibiting vasodilation. The simultaneous release of erythrocyte arginase during hemolysis may limit the availability of arginine to NOS, contributing to a deficiency of NO in vascular system (18).

Aslan and Freeman have shown that superoxide radical formed in the reaction catalyzed by xanthine oxidase in the endothelium inhibit the action of NO in the vasculature of transgenic sickle cell mice (19, 20). Overproduction of ROS in microvasculature increases the oxidative stress that can disrupt NO homeostasis and produce the highly oxidative peroxynitrite radicals. Our finding of increased level of NO in SCD patients is further supported by a previous study reporting accelerated auto-oxidation, enhanced oxidative stress, increased susceptibility to lipid peroxidation, and increased generation of ROS in sickle cell patients (4, 7).

Essien et al have found significantly low values of plasma vitamins A (retinol), C (ascorbic acid) and E (alpha tocopherol) in patients with sickle cell anemia in steady state compared to controls, which support our results (21). Vitamin E has chain breaking antioxidant, membrane protective and anti-inflammatory actions. While vitamin C synergistically act together with vitamin E by spearing its action. Studies performed with mixed tocopherols supplementation have demonstrated that vitamin E activates endothelial NOS, increases NO release, and decreases platelet aggregation *in vivo* (22). Adelekan and Ray et al, who also noticed significantly low levels of antioxidant vitamins such as β-Carotene, Vitamin E and Vitamin C in sickle cell patients (6, 23).

Regular supplementation of these vitamins may ameliorate some of the sickle cell manifestations such as vasoocclussive crises, acute chest syndrome, recurrent infection and growth retardation (2). Natta et al observed marked reduction in the number of circulating irreversible sickle erythrocytes in their 35 week supplementation of vitamin E in patients with sickle cell anaemia (24). Supplementation of antioxidant vitamins can mitigate the oxidative stress which may improve the bioavailability of NO, vasodilation and prevent vasoocclussive crisis.

Thus, our study shows elevated serum NO and depleted antioxidant vitamins suggests hyphened oxidative stress in SCA patients. We conclude from this study that the regular antioxidant supplementation might be useful in the management of SCA patients.

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CARDIORESPIRATORY CHANGES WITH COMPACT BACKPACK SYSTEM AND DISTRIBUTED MODE OF LOAD CARRIAGE

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Abstract : In Indian Army, soldiers normally carry 21.4 kg in backpack (BP), haversack, and web distributed in different parts of the body and rifle in hand. This load distribution is unequal, may involve excess energy expenditure, mostly uncomfortable, and restricts the normal movement of the hand carrying rifle. A new BP has been developed which accommodates the rifle on sides leaving the hands free. Physiological evaluation of load carriage [21.4 kg in the existing Load Carriage ensembles (LCe) and in the new BP] and without load was carried out on a group of Indian Army soldiers (n= 8) to understand the efficacy of the new BP vis-á-vis the existing one at 4.5 km/h speed at level ground and at 5% gradient on a treadmill in controlled laboratory environment. Heart rate, oxygen consumption, relative work load and energy expenditure were determined and one-way repeated measure ANOVA was applied to compare the results. All the physiological parameters showed higher responses in distributed mode in comparison to compact mode. However, the differences were not significant. The study may be carried out on a larger sample size to find out the better efficacy of compact mode of load carriage over the distributed mode.

Key words : load carriage compact mode distributed mode

INTRODUCTION

Carrying moderate to heavy load is a common phenomenon in military operations and many industrial setups. In Indian Army, soldiers have to carry loads ranging from 20 to 30 kg in different terrains and extreme environmental conditions. The composite load in existing Load Carriage ensembles (LCe) amounts to be 21.4 kg and consists of backpack (BP, 10.7 kg), haversack (HS, 4.4 kg) and web (2.1 kg) distributed in different parts of body and INSAS rifle (4.2 kg) in hand. This load distribution is unequal and may cause problems in the body of the user. The LCe contains specific items as per the requirement of soldier for different operations. BP is placed at the back while

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web is tied in front of the body. Weight distribution in this combination of frontpack (FP) and backpack (BP) is unequal. According to previous researchers the FP – BP combination becomes most economical in terms of energy cost when equal load is given in both sides of the body (1). Placement of HS on the body is not fixed. Sometimes it is placed on the either side of the body or attached in the lower portion of the back with the belt at the bottom of the BP. These arrangements are mostly uncomfortable to the user. Carrying rifle in right hand restricts normal swinging motion of the said arm. Birrel and Haslam (2) showed that restricted arm movement caused body's centre of mass to deviate from its normal path which might lead to excess energy expenditure.

Different modes of load carriage have been thoroughly investigated by several researchers in the past (3-13). Considerable research has been carried out to determine best method of load carriage that minimized the physical stress on the body (1, 7, 14-17). But the studies on physiological effects of distribution of load in different parts of the body are very few (18-20).

In most of the earlier studies load was placed as single unit (CM) such as BP. Pal et al (21) compared the effect of carrying 10.7 kg load in compact BP and distributed mode (DM) and found that physiological cost was more in DM than in CM during level walking. The present study was designed to evaluate the cardiorespiratory responses of carrying standard 21.4 kg military load in two different modes (CM and DM) at 4.5 km/h walking speed in two different gradients by Indian soldiers.

METHODS

Eight physically fit male soldiers of Indian army without any history of musculoskeletal disorders or cardiovascular pathology and with a service experience of atleast four years volunteered for this study. Their mean (SD) age, height, weight and maximum aerobic capacity (VO_{2max}) were 29.83 (2.86) yrs, 165.5 (3.15) cm, 63.5 (5.47) kg and 31.88 (4.13) ml/min/kg. respectively. They signed informed consent before participating in the experimental procedure.

Experimental details

A clearance from the Ethical Committee was obtained for the study. Thereafter, soldiers were briefed about the purpose and the risk of the study. Initially, they were allowed to walk on treadmill (Taeha, Intertrack 6025, Korea) for habituation at various speeds without and with loads at different gradient in the laboratory. After that maximum oxygen consumption (VO_{2max}) of the subjects was measured during treadmill exercise with regular increase in the gradient (Harbor protocol, 22), keeping the speed constant. During the measurement of VO_{2max} subjects wore vest, underwear, shorts and physical training shoes. On the day of experiment all the subjects reported to the laboratory at 0800 hrs after light breakfast. They were allowed to take rest for one hour before the commencement of experiment. Subjects were debarred from smoking and taking any food till they were in the laboratory. During the experiment subjects wore full Indian Army combat uniform including combat boot (weighing 2.5 kg). Load carriage experiments were carried out on each subject with 21.4 kg load

(33.5% of body weight) in two different modes (CM and DM) and without load (NL) at 4.5 km/h walking speed and at two gradients (0 and 5%) on treadmill for 10 min duration. Distributed mode (Fig. 1) of load comprised of BP (10.7 kg) on the back, HS (4.4 kg) in the waist region, web (2.1 kg) tied in front

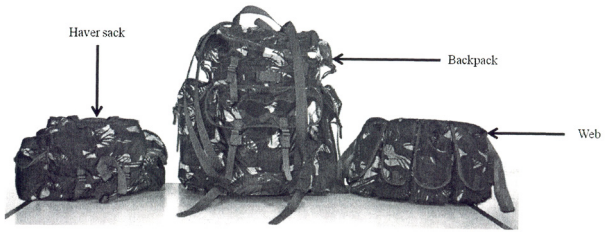

Fig. 1: Existing Load Carriage Ensembles.

at abdomen and INSAS rifle (4.2 kg) in hand. Compact mode involved carrying 21.4 kg load with all belongings of the DM including rifle fitted into newly designed larger BP (CM) (Fig. 2a, b). The mode, magnitude and placement of the loads are given in Table I. A total of 48 experiments (3 Loads × 2 Grades × 8 subjects) were performed. Each subject was required to complete two conditions

Fig. 2a: Newly designed backpack (Front view).

Fig. 2b: Newly designed backpack (Back view).

per day (between 0930 hrs to 1300 hrs), with atleast 60 min rest between two experiments.

Cardiorespiratory measurements

All load carriage experiments were conducted in controlled laboratory environment at 22°–25°C, 50–55% relative humidity, at same hours of the day between 0930 hrs and 1330 hrs for eliminating specific dynamic actions of food for all practical purposes. During the experiments, heart rate (HR), oxygen uptake (VO_2), relative work load ($\%VO_{2max}$) and energy expenditure (EE) of each of the individuals were determined by the process of breath by breath gas analysis using $K4b^2$ system ($K4b^2$, Cosmed, S.r.l, Italy). Average of the last 3 minutes HR, VO_2, $\%VO_{2max}$ and EE data of 10 min walking trial were considered as individual value and subjected to statistical treatment.

Statistical analysis

A descriptive statistics in the form of

TABLE I: The mode, magnitude and placement of load during load carriage experiment.

Condition	*Weight (kg)*	*Placement of load*	*Mode*	*% of body (kg) weight*
NL	0.0	No load	–	–
DM	21.4	10.7 kg BP on the back, 4.4 kg HS in the waist, 2.1 kg web in front near abdomen and 4.2 kg Rifle in hand.	Distributed mode	33.5%
CM	21.4	Modified larger BP	Compact mode	33.5%

mean and standard deviation is presented in table II for various cardiorespiratory parameters e.g HR, VO_2, $\%VO_{2max}$ and EE.

An one-way repeated measure ANOVA was applied as the same subjects were used for NL, DM and CM conditions at 0% and 5% gradient to see overall significance across the conditions. Followed by the significance observed for the various cardiorespiratory parameters across the conditions mentioned above a Bonferroni Post-Hoc. test was applied to compare between the conditions pair wise. For all the tests statistical significance were verified at $P<0.05$ level.

RESULTS

The results revealed that the significant changes in HR [$F_{(1.14, 8.04)} = 19.61$, $P<0.05$ at 0% gradient; $F_{(2, 14)} = 226.41$, $P<0.05$, at 5% gradient], VO_2 [$F_{(1.06, 7.43)} = 17.10$, $P<0.05$ at 0% gradient; $F_{(1.08, 40.01)} = 28.61$, $P<0.05$ at 5% gradient], $\%VO_{2max}$ [$F_{(1.04, 7.33)} = 13.76$, $P<0.05$ at 0% gradient; $F_{(1.09,7.67)} = 28.90$, $P<0.05$ at 5% gradient] and EE [$F_{(1.15, 8.11)} = 37.30$, $P<0.05$ at 0% gradient; $F_{(1.05, 7.41)} = 65.27$, $P<0.05$, at 5% gradient] across NL, DM and CM conditions at 0% and 5% gradient (Table II).

After performing Bonferroni Post-Hoc, test significant increase in HR was observed for DM (21.07% at 0% and 30.92% at 5% gradient, $P<0.05$) and CM (18.15% at 0% and 27.06 % at 5%, $P<0.05$) in comparison to NL at 0% and 5% gradients. However, the increase in HR was found to be insignificant when DM and CM were compared at 0% (2.46%) and 5% (3.10%) gradient.

TABLE II: Mean±SD of different physiological parameters in three modes and two gradients of load carriage at constant speed (4.5 kmph).

Parameters	*Gradients (%)*	*Load*			*% Increase*		
		NL	*DM*	*CM*	*NL vs DM*	*NL vs CM*	*DM vs CM*
HR	0	92.8±7.05	112.3±13.97	109.5±12.69	21.07*	18.15*	2.46^{NS}
(Beats/mm)	5	105.7±10.24	137.9±9.06	133.8±9.90	30.92*	27.06*	3.10^{NS}
VO_2	0	11.63±2.35	16.07±4.01	15.54±4.29	38.67*	33.18*	4.23^{NS}
(ml/min/kg)	5	16.20±2.80	22.03±5.16	21.38±4.87	35.26*	31.37*	2.96^{NS}
$\%VO_{2max}$	0	35.47±6.26	49.44±14.17	47.84±14.97	38.67*	33.18*	4.23^{NS}
	5	49.62±8.87	67.40±16.21	65.56±15.53	35.26*	31.37*	2.96^{NS}
EE	0	3.98±0.65	5.28±0.80	5.11±0.89	33.72*	28.98*	3.81^{NS}
(Kcal/min)	5	5.47±0.65	7.36±0.95	7.12±1.02	34.73*	30.11*	3.65*

*P<0.05, NS = Not significant.

The increase in VO_2 was found to be significant for DM (38.67% at 0% gradient and 35.26% at 5% gradient, P<0.05) and CM (35.26% at 0% gradient and 31.37% at 5% gradient, P<0.05) in comparison to NL at 0% and 5% gradient. However, the increase in VO_2 was found to be insignificant when DM and CM were compared at 0% (4.23%) and 5% (2.75%) gradient.

The increase in $\%VO_{2max}$ was found to be significant for DM (38.67% at 0% gradient and 35.26% at 5% gradient, P<0.05) and CM (33.18% at 0% gradient and 31.63% at 5% gradient, P<0.05) in comparison to NL at 0% and 5% gradient. However, the increase in $\%VO_{2max}$ was found to be insignificant when DM and CM were compared at 0% (4.23%) and 5% (2.96%) gradient.

There was a significant increase in EE for DM (33.72% at 0% gradient and 34.73% at 5% gradient, P<0.05) and CM (28.98% at 0% gradient and 30.11% at 5% gradient, P<0.05) in comparison to NL at 0% and 5% gradient. At 5% gradient the increase in EE was found to be significant (3.65%, P<0.05) when DM and CM were compared. However, the increase was found to be insignificant (3.81%, P<0.05) at 0% gradient for the same comparison.

DISCUSSION

The present study was conducted to explore whether any differences exist in physiological responses while carrying the same magnitude of the load in the DM and CM. Results showed overall significant changes in all four physiological parameters recorded across NL, DM and CM. Similar finding was observed by Soule et al (23) who found that the demands of energy cost during load carriage probably depend on the pattern of load distribution. If the load is well distributed, balanced and placed close to the centre of the body it demands less energy cost than load in unbalanced positions. Results of their study revealed a lower energy cost when carrying the load in the CM. Malhotra and Sengupta (5) conducted load carriage experiment on school children (carrying school bags weighing 6.0 lb in four different position, i.e. rucksack, low back, across the shoulders and hands) to identify the most economical way of carrying school bags by them. They concluded that rucksack was the most economical and efficient, whereas the hand carriage was the most inefficient method in terms of energy expenditure for Indian children. Bonferroni Post-Hoc. test revealed that there were significant increase in all the physiological parameters from condition NL to conditions DM and CM, but the increases were not significant when conditions DM and CM compared, except the EE at 5% gradient which showed a significant increase for condition DM than condition CM (Table II). In previous studies (6, 7) the principle of keeping the load close to the trunk was followed by placing it in a CM (e.g. double pack). In the present study the CM is a single unit which utilizes the large muscle mass of back and trunk. At the same time arms are left free to swing normally to maintain the centre of mass of the body in its utmost position for minimum energy expenditure. This arrangement allowed the body to move in a more balanced way compared to the DM.

Rifle carriage in hand may be considered as an isometric work. Jackson et al (24)

showed that when an isometric exercise component was added to a dynamic exercise task, cardiovascular responses were elevated above levels noted for the dynamic exercise alone. In the existing DM, INSAS rifle is carried in hand that disturbs the balance and normal swing of arm during load carriage. Graves (25) compared between hand weight, wrist weight and ankle loads and found 1.36 kg increase in hand or wrist weight increases the energy cost more than ankle weight and provided additional exhaustion to the upper body. Other researchers have also found that load carriage in hand is among the worst when compared between modes of load carriage (6, 7). Birrel et al (26) studied the effect of military load carriage on ground reaction force. They found rifle carriage and restriction of natural swing changed vertical and horizontal position of the body's centre of mass. Birrel and Haslam (2) showed restricted arm movement due to rifle carriage in one hand causes increased range of motion of body's centre of mass. Extra energy may be required to normalize centre of mass in this situation. Greater muscular activity of the arm and shoulder carrying rifle may be the key factor behind excess physiological cost with distributed mode in our study. This observation is in line with the present study where there was a significant increase in EE at 5% gradient with DM compared to the CM.

In the existing load carriage system (DM), BP is attached to an outside metallic frame to hold it properly. This frame has fixed dimensions, thus less compatible to major population group of the Indian Army. In the newly designed CM, the BP is provided with a telescopic frame inside. This arrangement allows the user to adjust the frame according to the length of their back. Compact mode has other features like additional waist strap which holds the bag close to the body so that the centre of mass does not move away. The changes in all physiological parameters were less in CM compared to DM. The result of the study indicates that CM as the better mode of load carriage than DM. The study may be carried out on a large number of subjects for further verification of the efficacy of CM over DM.

The knowledge of this study will help in design and development of new load carriage ensembles to reduce the cardiorespiratory burden during load carriage.

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EFFECT OF GARLIC *(ALLIUM SATIVUM)* ON HEMATOLOGY AND ERYTHROCYTE ANTIOXIDANT DEFENSE SYSTEM OF ALBINO RATS EXPOSED TO HEAVY METALS (NICKEL II & CHROMIUM VI)

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Abstract : Heavy metals are stable environmental contaminants, causing various alterations in target tissues. Garlic has some beneficial effect in preventing heavy metal induced various alteration. The objective was to investigate the possible protective role of fresh aqueous homogenate of garlic on hematology, erythrocyte antioxidant defense system in male albino rats treated with $NiSO_4$ and $K_2Cr_2O_7$. Rats were divided into six groups. Group I was untreated control. Group II was given aqueous homogenate of garlic (orally). Group III was administered with nickel sulfate (i.p). Group IV was given $NiSO_4$ and garlic simultaneously. Group V was administered with $K_2Cr_2O_7$ (i.p). Group VI were treated simultaneously with $K_2Cr_2O_7$ and garlic. RBC, WBC, platelet count, PCV%, hemoglobin concentration decreased significantly and clotting time increased significantly after nickel treatment. After chromium treatment all the values decreased except clotting time. Increased malondialdehyde and glutathione level after nickel and chromium treatment was observed. Also erythrocyte superoxide dismutase, glutathione peroxidase and catalase activities significantly increased after nickel and chromium treatment. Simultaneous garlic supplementation exhibited protective role to combat nickel toxicity, whereas no such beneficial effects were observed for chromium (VI). Garlic may partially prevent nickel and chromium induced alteration but such ameliorated effects as an antioxidant is only restricted on nickel induced alteration.

Key words : allium sativum, nickel II, chromium VI, hematology, erythrocyte antioxidant defense

INTRODUCTION

Heavy metals are stable and persistent environmental contaminants and have the potential to cause various alterations in target tissues of exposed humans. Hence the

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environment, in which a person works, can be a major factor in determining health. Heavy metals are trace metals with a density at least five times that of water. As they cannot be metabolized by the body, these metals are bio accumulative. Among these, nickel and chromium are used extensively in stainless steel industry, the single largest industry, along with welding and electroplating industry. As a result, occupational exposures to these are very high via inhalation or ingestion of metal containing diet. After entering into the body, both nickel and chromium penetrates all organs but mainly accumulates into liver, kidney, lungs, bone etc (1, 2). Nickel can induce severe liver and kidney damage by altering several marker enzymes and ascorbate cholesterol metabolism along with histopathological alterations (3, 4). On the other hand, hexavalent chromium has been reported to cause hepatotoxicity in both human and laboratory animals (5). There is also evidence of progressive lung cancer with exposure to chromium (VI) in chrome pigment industry workers (6). Evaluations of toxicity of the metals are facilitated by results of basic hematological assays (7). Nickel and chromium induced alteration of hematological parameters were reported earlier (1, 2). Nickel can affect erythrocyte membrane lipid bilayer and membrane protein (8). It is well documented that heavy metals usually bind with the erythrocyte membranes and plasma albumin and stimulates metallothioneins and ROS. This results in oxidative damage in erythrocyte and in various tissues (9, 10). Erythrocytes are equipped with defense system representing their antioxidant capacity. This system includes glutathione and other enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) (11).

Garlic *(Allium sativum)* has played an important dietary and medicinal role throughout the history of mankind. The therapeutic efficacy of garlic encompasses a wide variety of ailments including cardiovascular, cancer, hepatic and microbial infection (12). Previous studies in our lab showed that garlic has some beneficial effect in preventing nickel and chromium induced alteration of serum lipid profile (13). All these are attributed to the presence of various organo sulfur compounds in garlic which are having tremendous antioxidant property (14). As our previous studies and various other studies have revealed the beneficial effect of aqueous homogenate of raw garlic, the present study was designed to investigate the possible protective role of fresh aqueous homogenate of garlic on some hematological parameters, erythrocyte antioxidant defense system and lipid peroxidation in male albino rats treated with nickel sulfate (NiSO4) and potassium dichromate (K2Cr2O7).

MATERIALS AND METHODS

Adult (aged 60-70 days) laboratory bred male Wister rats weighing 160±5 g each were fed with laboratory stock diet and water *ad libitum* for 7 days. The acclimatized animals were divided into six groups of six animals each. Group I served as an untreated control. Group II were administered aqueous homogenate of fresh raw garlic at a dose of 250 mg/kg b.wt; orally (15). Group III was treated with nickel sulfate (New India Chemical Enterprises, Kochi, India) in double-distilled water at a dose of 2.0 mg/100 g b.wt, intraperitoneally (i.p.) (16). Group IV rats received both nickel sulfate (2.0 mg/100 g

b.wt, i.p) and garlic (250 mg/kg b.wt; orally) simultaneously. Group V rats were given potassium dichromate (Qualigens Fine Chemicals, Mumbai, India) in double distilled water at a dose of 0.5 mg/100 g b.wt, intraperitoneally (i.p.) (17). Group VI rats were treated simultaneously with potassium dichromate (0.5 mg/100 g b.wt, i.p.) and garlic (250 mg/kg b.wt; orally). Nickel sulfate and potassium dichromate were given on alternate days until tenth dose whereas garlic was administered orally everyday until twentieth dose. The entire experimental protocol was approved by Institutional ethical committee and utmost care was taken during the experimental procedure, as well as at the time of sacrifice, according to ICMR guidelines (18). After the treatment, the animals were sacrificed by decapitation always between 9:00 h and 11:00 h and fresh blood was immediately collected into heparinized test tubes.

Determination of hematological parameters

All the hematological parameters i.e. total red blood corpuscles (RBC) count, packed cell volume (PCV), total white blood corpuscles (WBC) count and total platelets count were measured by using fully automated hematology analyzer (Sysmax K-4500). The hemoglobin (Hb) concentration in the red cell lysates was also determined by the using fully automated hematology analyzer (19).

Biochemical determination of antioxidant status of erythrocyte

Blood samples were centrifuged at 3000 rpm for 15 min to remove the plasma and buffy coat. The erythrocytes were washed three times in buffered saline (0.9% saline in 0.01 M phosphate buffer, pH 7.4), and the packed cells were suspended in equal volume of the buffered saline. Erythrocyte lipid peroxide (LPO) was measured as the production of malondialdehyde (MDA) which in combination with thiobarbituric acid (TBA) forms pink chromogen compound whose absorbance at 530 nm was recorded. The concentration of MDA (nmol/g Hb) was calculated using a standard curve obtained from the reaction between varying MDA concentration (20). The Total (Cu-Zn and Mn) superoxide dismutase activity was determined according to the method of Misra and Fridovich (21). The ability of superoxide dismutase to inhibit the auto oxidation of epinephrine at pH 10.2 has been used as the basis of a convenient and sensitive assay for this enzyme. One unit of SOD activity was defined as the amount of enzyme that inhibited the oxidation of epinephrine by 50%. Activity was expressed as units/mg of protein. It was expressed in units of enzyme activity per gram of hemoglobin (Unit/g Hb). Total erythrocyte glutathione (GSH) was estimated by the method of Beutler et al (22). Estimation of erythrocyte glutathione peroxidase (GSH-Px) was done by the method of Paglia and Valentine (23). GSH-Px catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH) the oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to NADP±. The decrease in absorbance at 340 nm was measured and the activity was expressed as Unit/g Hb. Catalase (CAT) activity in hemolysate was assayed by the method of Aebi (24). Catalase decomposes the H_2O_2 and forms water and molecular oxygen. H_2O_2 absorbs maximum

light at 240 nm. When H_2O_2 is decomposed by catalase, the absorbance is decreased. The decrease in absorbance was measured at 240 nm at an interval of 15 seconds for one minute. The difference in absorbance (DA at 240 nm) per unit time was a measure of the catalase activity. The catalase activity was expressed as mM of H_2O_2 decomposition/ microgram Hb/min.

Mean±SD values were calculated for each group. To determine the significance of inter-group differences, we analyzed each parameter separately. A one way analysis of variance (ANOVA) followed by post hoc't' test was done to determine which of the groups differed among themselves using statistical software (StatPac for Windows, Version 11.0)

RESULTS

Table I shows that intraperitonial nickel sulfate administration resulted in significant decrease in red blood cell (RBC) count, white blood cell (WBC) count, hematocrit value (PCV%) and haemoglobin (Hb) concentration when compared to that of control group. Simultaneous garlic administration also showed a significant increase in the above parameters from nickel treated group. A significant increase of clotting time (CT) and decrease in platelet count in nickel treated group was observed when compared to untreated control. But simultaneous garlic administration was unable to improve the above parameter significantly. Similarly, after treatment with potassium dichromate, RBC count, WBC count, Hb concentration and PCV% values were decreased significantly from untreated control value but no such changes are noticed in case of CT after chromium treatment (Table I). Garlic administered simultaneously to chromium treated rats was significantly able to bring

TABLE I: Effect of Garlic on hematological parameters after nickel sulfate and potassium dichromate treatment in rats.

Treatment Group	*Group I*	*Group II*	*Group III*	*Group IV*	*Group V*	*Group VI*	*F-ratio & P value*
RBC (10_6 cells/µl)	8.468± 0.974	9.132± 1.031	4.932± 0.727******###	6.537± 1.310******###$	6.165± 0.615******###$	7.630± 0.831***#$$+@	F=16.44 P=0.000
Hemoglobin (gm/dl)	17.57± 1.07	19.35± 1.07	11.05± 1.75******###	13.49± 1.60******###$	13.62± 1.47******###$	15.50± 1.51***###$$+@	F=26.52 P=0.000
PCV (%)	49.21± 2.34	50.14± 2.06	41.57± 2.32****###	46.80± 1.30$	43.87± 1.96*#	44.56± 3.75*#	F=11.27 P=0.000
Clotting Time (min)	4.21± 0.34	4.49± 0.32	7.53± 0.77***#	7.23± 0.66***#	4.78± 0.78$$++	4.65± 0.63$$++	F=35.29 P=0.000
Platelets (10_3 cells/µl)	866.87± 61.02	758.41± 37.61*	523.33± 68.31****###	566.66± 53.54****###	593.33± 57.15****###	586.66± 70.61****###	F=30.62 P=0.000
WBC (10_3 cells/µl)	8.63± 0.42	9.30± 0.52	4.91± 0.34****###	6.20± 0.52***$	6.03± 0.50***$	7.59± 079***#$$+@	F=59.77 P=0.000

Treatment groups: I - untreated control; II - garlic; III - nickel sulfate (NiSO4); IV - nickel sulfate + garlic; V - potassium dichromate ($K_2Cr_2O_7$); VI - potassium dichromate + garlic. Each value is mean±SD of six observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tuky-Krammer test. The * depicts comparison with Group I (*P<0.05, **P<0.01, ***P<0.001); # depicts comparison with Group II (#P<0.05, ##P<0.01, ###P<0.001); $ depicts comparison with Group III ($P<0.05, $$P<0.01, $$$P<0.001); + depicts comparison with Group IV (+P<0.05, ++P<0.01, +++P>0001); @ depicts comparison with Group V (@P<0.05).

the changed value of RBC, WBC and Hb concentration towards that of control value. Although we have found a significant decrease in platelet count in chromium treated rats, but garlic administration was unable to improve the situation. In rats, treated with only garlic homogenate, there was no significant change in the above parameters except a significant fall in platelet count.

Fig. 1 shows the percent change decrease of RBCs count, PCV% and Hb concentration in group IV and group VI rats in comparison to the untreated control Group I. But when compared with group III and group V in relation to group I respectively, the decrease of RBCs count, PCV (%) and Hb concentration were found to be remarkably less [E-2 vs. E-3 and E-4 vs. E-5]. Fig. 1 also

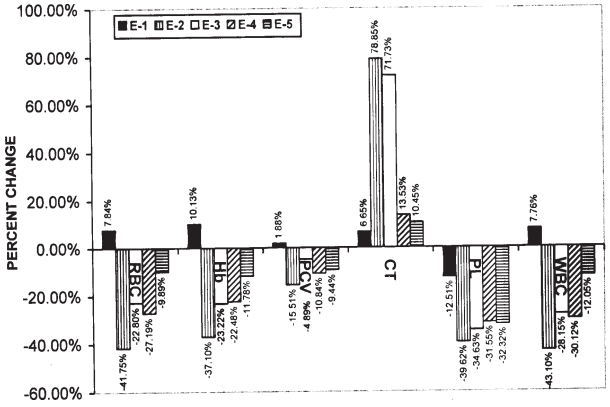

Fig. 1 : Percent change chart of various hematological parameters of different study groups after administration of nickel sulfate and potassium dichromate either alone or in combination with aqueous garlic extract as compared to untreated control group in male rats. RBC, red blood corpuscles; Hb, hemoglobin; PCV, packed cell volume; CT, clotting time; WBC, white blood corpuscles; PL, platelet. E-1, Group I (control) vs. Group II (+garlic); E-2, Group I vs. Group III (+$NiSO_4$); E-3, Group I vs. Group IV ($NiSO_4$ + garlic), E-4, Group I vs. Group V (+ $K_2Cr_2O_7$); E-5, Group I vs. Group VI ($K_2Cr_2O_7$ + garlic).

shows the percent change increase of clotting time, decrease of platelet count and WBCs count in group IV and group VI rats when compared to untreated control (group I). But when it was compared with group III (only nickel treated) and group V (only chromium treated) in relation to group I the change in all the parameters were surprisingly less [E-2 vs. E-3 and E-4 vs. E-5]. In the red blood cells of the nickel sulfate and potassium dichromate treated rats malondialdehyde (MDA), glutathione (GSH) levels and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were significantly increased when compared to untreated control (group I) rats (Table II). Simultaneous treatment with garlic and nickel sulfate reversed this change significantly towards control value. We have not observed any significant improvement in all the above parameters in garlic and chromium treated rats (group VI) from that of chromium treated group (group V) (Table II).

Fig. 2 shows the percent change of anti-oxidant status of erythrocyte lysates in different groups in comparison with group I control. It shows the percent increase of erythrocytes MDA and GSH contents and the activities of erythrocyte SOD, GSH-Px and CAT in group IV ($NiSO_4$ ± garlic) and group VI (K_2Cr_2O ± garlic) rats in comparison to group I. But when compared with group III receiving nickel sulfate only, the rise in group IV rats in relation to group I is found to be amazingly less [E-2 vs. E-3]. Rats receiving garlic extract (group II) did not show any significant variation in the antioxidant parameters studied above when compared with untreated control (group I).

TABLE II : Effect of Garlic on Erythrocyte Lipid peroxidation and antioxidant enzyme activities after nickel sulfate and potassium dichromate treatment in rats.

Treatment Group	*Group I*	*Group II*	*Group III*	*Group IV*	*Group V*	*Group VI*	*F-ratio & P value*
MDA (nmoles/g Hb)	226.95± 23.28	228.11± 31.59	334.39± 33.10**	289.96± 44.60*"$	323.56± 35.35**+	312.96± 26.73**	F=12.38 P=0.000
Glutathione (µmoles/g Hb)	19.52± 4.81	19.92± 1.75	36.05± 3.24***#	22.83± 4.03$$	36.38± 6.15***#++	34.28± 5.66***#+	F=19.83 P=0.000
GSH-Px (units/g Hb)	75.24± 9.72	73.44± 8.08	189.95± 25.80***###	117.88± 45.78***#$$	195.73± 32.15***###++	174.01± 18.46***###+@	F=26.54 P=0.000
SOD (units/g Hb)	1258.62± 111.38	1267.20± 58.35	1901.78± 302.82***#	1566.87± 458.85**$	1845.33± 307.69***++	1800.83± 323.11***+	F=5.84 P=0.001
CAT (units/g Hb)	897.12± 181.74	825.60± 103.64	1235.4± 50.36*##	956.18± 152.39$	1333.41± 132.85*##+	1191.83± 120.00*#+@	F=15.17 P=0.000

Treatment groups: I - untreated control; II - garlic; III - nickel sulfate (NiSO4); IV - nickel sulfate + garlic; V - potassium dichromate ($K_2Cr_2O_7$); VI - potassium dichromate + garlic. Each value is mean±SD of six observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tuky-Krammer test. The * depicts comparison with Group I (*P<0.05, **P<0.01, ***P<0.001); # depicts comparison with Group II (#P<0.05, ##P<0.01, ###P<0.001); $ depicts comparison with Group III ($P<0.05, $$P<0.01, $$$P<0.001); + depicts comparison with Group IV (+P<0.05, ++P<0.01, +++P>0001); @ depicts comparison with Group V (@P<0.05).

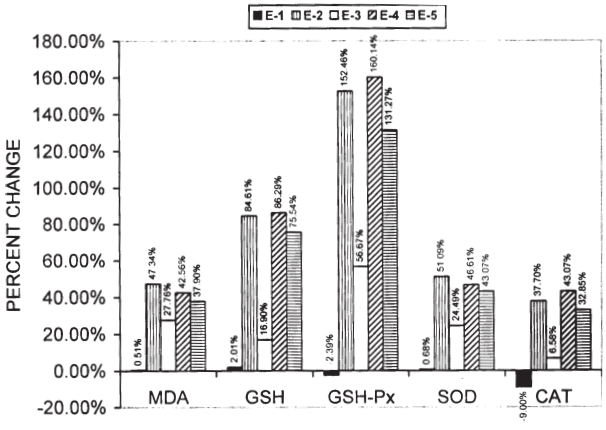

Fig. 2 : Percent change chart of antioxidant status in red cell lysates of different study groups after administration of nickel sulfate and potassium dichromate either alone or in combination with aqueous garlic extract as compared to untreated control group. MDA, malondialdehyde; GSH, glutathione; GSH-Px, glutathione peroxidase; SOD,superoxide dismutase; CAT, catalase. E-1, Group I (control) vs Group II (+ garlic); E-2, Group I vs Group III .(+$NiSO_4$); E-3, Group I vs Group IV ($NiSO_4$ + garlic),E-4, Group I vs Group V (+$K_2Cr_2O_7$); E-5, Group I vs Group VI ($K_2Cr_2O_7$ + garlic).

DISCUSSION

From our observation, it can be seen that, treatment with either nickel sulfate or potassium dichromate induces anemia type condition (decreased RBC count, PCV and Hb concentration) in rats although both nickel and chromium are essential trace element for normal physiological functioning of our body. Previous studies have shown that nickel may adversely affect hematopoiesis (25). In our study, decrease in RBC count, PCV% and Hb concentration may be due to non-regenerative anemia arising due to nickel induced direct injury of hematopoietic stem cells resulting in decreased RBC count, WBC count, Platelet count (26). In this study, decrease in platelet count occurred may be due to decreased production or increased consumption of them. Alterations of WBC count and decreased hematocrit value in rats have already been reported following

administration of nickel (27). Nickel sulfate may depress bone marrow activity and significantly decrease all the types of blood cells, as per our observation in our previous study (26). Further study revealed that chromium (VI) exposure to rats resulted in reduction of RBC count and hematocrit value along with a decrease in haemoglobin concentration (28). The decrease in hemoglobin appears to be due to inhibition of its biosynthesis by decreasing the succinyl pool as well as glycine pool. Both are required in the initial stage of the heme biosynthesis (29). Simultaneous treatment with garlic decreased the toxic effect of both nickel and chromium by showing a protective role in anemia and leucopenia. This may be due to the stimulation of bone marrow activity. Interestingly simultaneous treatment with garlic was found to be incapable of improving either nickel or chromium induced thrombocytopenia. A significant decrease in platelet count was observed in case of garlic treatment alone which may be due to dual properties of garlic i.e. antioxidant as well as pro-oxidant activities. It is also known that, sulfur compound in garlic significantly prolong bleeding time and thrombin time (30).

Reactive oxygen species (ROS) include free radicals and non-radical species like superoxide and hydroxyl radical and hydrogen peroxide, singlet oxygen etc. ROS are implicated as important pathologic mediators in many disorders. Increased generation of ROS and enhanced lipid peroxidation are considered responsible for toxicity of wide range of compounds (31). Our present study reveals that, treatment with either nickel sulfate or potassium dichromate increase lipid peroxidation by increasing MDA concentration in red blood cells of rats. This increase in MDA concentration was definitely accompanied by increased ROS formation (32). As a result, enhanced lipid peroxidation, DNA damage, altered calcium and sulfydryl homeostasis as well as marked disturbances in antioxidant defense system occurred (33). Simultaneous treatment with garlic was found to be effective in prevention of oxidative damage in erythrocyte, induced by nickel sulfate resulting in a significant decrease in erythrocyte MDA concentration but not so in case of potassium dichromate. The present study reported an increase in the level of GSH and the activities of GSH-Px, SOD and CAT enzymes in erythrocyte of rats treated with nickel or chromium. This change may be attributed to adaptive change. There is report that reduction of hexavalent chromium to trivalent chromium in erythrocyte occurs by the action of glutamione (34). It is also previously reported that oxidative stress increases the GSH-Px activity (35). In this study increased SOD activity indicates an increased production of superoxide radical in heavy metal (nickel II and chromium VI) toxicity. The increase in GSH-Px and CAT activity after nickel or chromium exposure may be explained by their influence on hydrogen peroxide as substrate, which is formed in the dismutation reaction of superoxide radical (36, 26). This action is followed by increased formation of GSH by glutathione reductase (37). Simultaneous garlic administration with nickel sulfate brought erythrocyte GSH level and activity of GSH-Px and CAT near to that of control value confirming a protective role of garlic extract on nickel induced impairment of erythrocyte antioxidant defense system. But such improvement was not found in rats treated

with potassium dichromate and garlic simultaneously. The cause may be due to a different pathway of action of chromium (VI). Effect of heavy metals on living tissues alters as per their atomic valence or elemental speciation. Hence considering elemental speciation in case of nickel as divalent metal and chromium VI which is hexavalent metal will definitely differentially act on the target cell (38). As a result of varying cellular response due to these toxic metals perhaps the simultaneous treatment of antioxidant like garlic also alters the degree of beneficial effects. We consider that further study is needed to evaluate the molecular mechanism in this pathway.

In conclusion It can be concluded from present findings that both nickel sulfate and potassium dichromate induced oxidative stress in erythrocyte leads to anemia, leucopenia and thrombocytopenia along with elevated erythrocyte MDA level and altered antioxidant defense system. Simultaneous treatment with aqueous homogenate of fresh garlic may partially protect against nickel and chromium induced toxicity on most of the hematological parameters but such ameliorative effect of garlic on erythrocyte antioxidant status were not noticed in case of hexavalent chromium induced toxicity in rats. Hence it may be considered that aqueous garlic homogenate is more useful protective antioxidant against nickel toxicities than chromium VI.

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ASSOCIATION OF ACYLATION STIMULATING PROTEIN WITH ENDOGENOUS SEX HORMONES & LIPID PROFILE DURING MENSTRUAL CYCLE

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Abstract : The study was done to investigate the effects of cyclical changes of endogenous sex hormones during different phases of menstrual cycle on Acylation stimulating Protein (ASP) and its correlation with lipid profile parameters in healthy reproductive women. Twenty nine healthy reproductive women with regular menstrual cycles were included in this longitudinal study. The levels of FSH, LH, progesterone and estradiol were measured along with ASP. The total cholesterol, triglycerides, HDL-C, LDL-C levels were estimated during follicular, ovulatory and luteal phases of the menstrual cycle. There was a significant rise in ASP levels during luteal phase when compared to follicular phase ($P<0.01$). The rise in ASP levels during the luteal phase correlated with elevated progesterone levels ($r=0.472$, $p=0.027$). Multiple regression analysis including all measured variables in the study showed that progesterone was the only significant predictor of ASP levels. The level of LDL-C as well as total cholesterol/HDL-C and LDL-C/HDL-C ratios showed significant decreases during the luteal phase compared with the follicular phase ($P<0.05$). No correlation was seen between ASP levels and the lipid profile parameters. The findings of this study suggest that adipokines such as ASP levels are increased during luteal phase associated with elevated progesterone levels which may contribute to increased fat storage & distribution in women of reproductive age.

Key words : sex hormones, acylation stimulating protein, luteal phase, lipid profile

INTRODUCTION

The menstrual cycle represents a continuous state of change in terms of female sex steroid environment. It is well established that fat storage and lipid

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metabolism are affected by hormonal changes in humans (1). Substantial amount of evidence indicates that sex steroids play a role in fat tissue regulation and distribution (2, 3). Differences in fat distribution and lipid profile exist between males and females of reproductive age. In females these changes coincide with onset of ovarian production of estrogen and progesterone during puberty (4-6) and with cessation of hormone production during menopause. Marked changes in their lipid metabolism occur during pregnancy & in reproductive disorders in response to hormonal changes (2, 3, 7). Investigations into the ability of estrogens and other sex hormones to alter plasma lipid and lipoprotein levels are important because these factors are significant indicators of cardiovascular risk in both men and women. Numerous studies have consistently shown the influence of exogenous sex hormones on lipid and lipoprotein levels, but studies of the effects of menstrual cycle phases on circulating lipid & lipoprotein levels have not shown a consistent pattern (8, 9). Due to the cycling nature of circulating levels of sex hormones in premenopausal women and their possible impact on levels of lipids and lipoproteins, and hence coronary heart disease risk, it is important to determine how these levels vary between the follicular and luteal phases of the menstrual cycle. This is especially important given that even small changes, such as may be seen between menstrual cycle phases, may be clinically relevant over a long period of time (10). The effects of menstrual cycle hormonal changes on factors linked to lipid metabolism are continuously investigated. These include studies on adipokines such as leptin, adiponectin and Acylation Stimulating Protein (ASP). Acylation Stimulating Protein is an autocrine hormone shown to affect lipid metabolism in humans and mice (11). It is produced through the alternate complement pathway by the interaction of complement factor C3 with factor B and factor D (also called adipsin), which results in the formation of C3a-des-Arg, also called ASP. In vitro, ASP increases triglyceride synthesis & storage in adipocytes through activation of diacylglycerol acyltransferase, the rate limiting enzyme in triglyceride synthesis and by stimulating glucose uptake (12, 13). The effect of ASP on fat storage is supported by some of the studies which have shown that ASP administration increases triglyceride clearance in mice and that ASP deficient (C3-/-) mice exhibit delayed postprandial lipid clearance & reduce adipose tissue depots (14-17). In humans ASP released from adipose tissue increases in coordination with triglyceride clearance (18). ASP levels decrease during fasting & after weight loss (19) and increase in obesity (20) and dyslipidemic disorders (21, 22). Limited evidence is available on effects of endogenous sex hormones on ASP and its correlation with lipid profile. Hence this study was aimed at investigating the effects of cyclical changes of endogenous sex hormones during different phases of menstrual cycle on ASP and its correlation with lipid profile parameters in healthy reproductive women.

MATERIALS AND METHODS

This was a longitudinal study involving 29 female subjects aged between 18-24 years. All of them had regular menstrual cycles of an average of 28 days, which was evident from the questionnaire provided to them during screening for enrolment. The women were considered healthy based on a medical

history questionnaire and routine blood investigations. They were not on any medications and had no disorders that may affect hormonal function such as polycycstic ovary syndrome. Women with irregular menstrual cycles & abnormally low hormone levels that suggest anovulatory cycles were excluded from the study. The study was approved by institutional ethics committee, and informed consent was taken from the subjects enrolled in the study. 2 ml of Peripheral venous blood sample was collected after overnight fasting on days 7, 14, & 21 days of the menstrual cycle. These days were chosen to represent different phases of an average 28 day menstrual cycle. Day 7 represented the midfollicular phase, day 14 represents the ovulatory phase & day 21 represents the midluteal phase. The blood samples were collected in plain tubes without anticoagulant for serum lipid and hormone estimation whereas EDTA tubes were used for ASP estimation. The serum was separated by centfifugation & stored at –80°C until analysis. The hormones that were estimated include Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), estradiol & progesterone. Estradiol, FSH & LH levels were measured by two-step immunoassays using Chemiluminiscent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as chemiflex TM, using system-architect i2000 (abbott laboratories). The progesterone assay was done using competitive immunoassay with direct Chemiluminiscent technology, ADVIA centaur system (Bayer Diagnostics). Samples were analysed for lipid profile parameters which included triglycerides, total cholesterol, Low Density Lipoprotein cholesterol (LDL-C) and High Density Lipoprotein cholesterol (HDL-C). Analysis was performed using automated Roche Integra 800's analyzer, Switzerland-Germany. The ASP estimation was done by using Sandwich ELISA technique. The human plasma EDTA samples, controls, and standard ASP were pretreated with polyethylene glycol 8000 to precipitate C3, thus preventing any artifactual generation of ASP. The samples were centrifuged, the supernatant was removed, and ASP was assayed immediately by an in-house ELISA using a monoclonal antibody as capture antibody and a polyclonal antibody as detecting antibody.

All the results were expressed as mean±standard error. Within – group differences at different phases were assessed by one way repeated measures analysis of variance (RM ANOVA). Pair wise comparisons were done by paired-t test to compare the means of variables at the different phases. Correlations between the measured variables in the different phases of the cycle were examined by bivariate analysis using pearson coefficients. For parameters with skewed distribution spearman correlation coefficient test was used. Logarithmic transformations were applied for parameters that did not follow a normal distribution. Stepwise multiple linear regression analysis was performed to determine the factors that significantly associated with variations in ASP levels. 'P' value <0.05 was considered as statistically significant. Analysis was done using SPSS version 11.0 for windows; SPSS, Inc, Chicago, IL.

RESULTS

All the 29 subjects had ovulatory cycle as judged by luteal progesterone levels above

5 ng/ml. General characteristics of the women included in this study are shown in table Liable 2 depicts the serum concentrations of endogenous sex hormones and ASP during follicular phase, midcycle and luteal phase of menstrual cycle. The fluctuations in estradiol, progesterone, LH and FSH levels are according to the expected levels in a typical regular 28 day cycle. Repeated measures ANOVA showed a significant overall change during the menstrual cycle for LH, FSH, progesterone and estradiol (P<0.0001). The ASP levels also showed significant changes during different phases of the cycle (RM ANOVA, P<0.05) as shown in Table II. There was a significant rise in ASP levels during midcycle (19.8±2.7nM) when compared to the follicular phase levels (14.6±1.8nM) (P<0.05). Similarly there was a significant rise in ASP levels during luteal phase (32.3±7.4) when compared to follicular phase (P<0.01). The increase in ASP levels during the luteal phase coincided with the increase in progesterone levels and progesterone/estrogen ratio in the luteal phase. Table III shows the levels of lipids and lipoproteins during follicular and luteal phases. There was no statistically significant differences in the levels of total cholesterol & triglycerides, between follicular phase &

TABLE I: Basic characteristics of subjects studied.

Parameter	*n*	*mean±SEM*
Age (yrs)	29	19.28±1.24
Weight (kgs)	29	52.37±11.35
Height (mts)	29	1.58±0.08
BMI (kg/m^2)	29	22.23±0.47

TABLE II: Concentrations of hormones & ASP during different phases of menstrual cycle.

Parameter	*Follicular phase*	*Midcycle*	*Luteal phase*	*'P'*
Estradiol (pg/ml)	25.72±1.60	105.3±11.5***	85.0±6.63$^{\#\#}$	0.002
Progesterone (ng/ml)	0.76±0.19	2.18±0.48**	7.18±0.76$^{\#\#\#}$	0.003
FSH (µIU/ml)	5.08±0.37	8.11±0.41**	3.19±0.32$^{\#\#}$	0.017
LH (µIU/ml)	3.42±0.25	13.40±2.24***	3.64±0.46$^{\#\#\#}$	0.038
ASP (nM)	14.6±1.8	19.8±2.7	32.3±7.4$^{\#\#}$	0.015

Values are expressed as mean±SEM.
*depicts comparison with follicular phase.
$^{\#}$depicts comparison with midcycle.
P<0.01,*P<0.001.
$^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001.

TABLE III: Lipid profile in different phases of menstrual cycle.

Parameter	*Follicular phase*	*Midcycle*	*Luteal phase*	*'P'*
Total cholesterol (mg/dl)	138.41±5.19	141.39±1.42	144.58±2.35	0.142
Triglycerides (mg/dl)	131.72±1.96	132.43±1.04	133.21±4.08	0.063
LDL-C (mg/dl)	142.33±3.08	108.6±2.76**	98.64±6.34$^{\#\#}$	0.015
HDL-C (mg/dl)	38.57±0.83	41.65±1.05	44.67±0.66	0.467

Values are expressed as mean±SEM.
*depicts comparison with follicular phase.
$^{\#}$depicts comparison with midcycle.
**P<0.01, $^{\#\#}$P<0.01.

luteal phase. There was slight increase in the levels of HDL-C during the luteal phase which was statistically not significant. The luteal phase measurement of LDL-C was significantly lower (p=0.01) compared with that during the follicular phase. The total cholesterol/HDL-C & LDL-C/HDL-C ratios were significantly lower [4.9% lower (p=0.006) and 7.9% lower (p=0.001) respectively] during the luteal phase compared to the follicular phase measurements. Table IV shows the correlation of ASP with hormones and lipids during the mid luteal phase. Increased ASP levels in the mid luteal phase showed a significant positive correlation with progesterone levels (r=0.472, p=0.027) and with progesterone/estrogen ratio (r=0.511, p=0.024). ASP also positively correlated with BMI (r=0.563, p=0.018). Setting plasma ASP (log transformed) as the dependent variable a multiple regression model was set to determine factors that predicted ASP levels in mid luteal phase. Hormones, lipids and BMI were entered into the model as predictors. The results showed that progesterone significantly associated with ASP levels (p=0.508, p=0.022) and entered this model as the only significant predictor.

TABLE IV : Bivariate correlation between ASP levels in the midluteal phase with other variables.

Variable	*r*	*P*
BMI	0.563*	0.018
Progesterone	0.472*	0.027
Estradiol	0.253	0.263
Progesterone/Estradiol ratio	0.511*	0.024
LH	–0.026	0.902
FSH	–0.362	0.143
Triglycerides	–0.934	0.702
Total cholesterol	0.291	0.236
LDL-C	0.333	0.187
HDL-C	0.122	0.642

*p=spearman correlation significant, two tailed.

DISCUSSION

In the present study we observed expected reproductive hormonal variations during different phases of an average 28 day menstrual cycle. An important finding of this study was that ASP levels changed significantly across the phases of menstrual cycle correlating with progesterone levels and progesterone/estrogen ratio. The increase in ASP levels correlated positively with the normally elevated progesterone levels in the mid luteal phase whereas no significant association of ASP was seen with estrogen levels or any other reproductive hormone. Multiple regression analysis including all measured variables in the study showed that progesterone was the only significant predictor of ASP levels. Our findings of increased ASP levels in the luteal phase of the menstrual cycle is in accordance with the findings of some of the recent studies on ASP. These studies showed that C3 and factor B (precursors of ASP) are produced in the human endometrium in a cyclic specific manner. It was found that luteal phase endometrium synthesize complement C3 de novo, whereas proliferative endometrium produces little or no C3. Likewise factor B, which is critical to the activation of the complement alternative pathway which leads to ASP production, has been shown to be present only in the luteal phase endometrium & not in the follicular phase. Also, factor B, was found to be synthesized in the endometrial cells of patients treated with exogenous progesterone therapy. Therefore, these precursors are found in the presence of high progesterone/estrogen ratio characterizing the luteal phase & not in the follicular phase which is marked with low progesterone & estrogen levels (23,

24). Although progesterone is considered to exert lipogenic effects in females, controversy still exists as to whether it mediates these effects directly or indirectly. Some studies have suggested direct anabolic effects or by effecting on insulin action, others suggest an action through specific transcription factors (25-27). This study has shown a link between levels of ASP, which is a potent fat storage factor, and progesterone which is known for its lipogenic effects in females. The significant positive association between luteal phase ASP and progesterone, as the main predictor of ASP levels in this study, may suggest a role for progesterone on ASP production and its lipogenic effects in healthy women of reproductive age.

The level of LDL-C as well as total cholesterol/ HDL-C & LDL-C/HDL-C ratios showed significant decreases during the luteal phase compared with the follicular phase. The slight increase in the level of HDL-C in the luteal phase, which may be clinically although not statistically, significant does indicate the exertion of an estrogenic influence on this lipoprotein level. No correlation was seen between ASP levels & the lipid profile parameters. However, small but highly significant decreases were seen in the luteal phase of the cycle which is consistent with previous studies that showed that lipid levels decrease during the luteal phase of the cycle explaining the anti atherogenic effects seen in females of reproductive age (9, 28). This may be associated with hormonal effects that enhance fat clearance from the circulation during that phase. Due to the cycling nature of circulating levels of endogenous sex hormones in women of reproductive age, and their possible impact on levels of lipids and lipoproteins, and hence Coronary Heart Disease (CHD) risk, it is important to determine how these levels vary between the follicular and luteal phases of the menstrual cycle. This is especially important given that even small changes, may be clinically relevant over a long period of time. These findings support our hypothesis of a lipid and lipoprotein profile that is associated with decreased risk of coronary heart disease during the luteal phase compared with the follicular phase of the menstrual cycle.

In conclusion ASP levels change significantly across the phases of the menstrual cycle and increase in ASP levels correlate positively with the normally elevated progesterone levels in the luteal phase. The progesterone is the main predictor of ASP levels emphasizing the role of progesterone on ASP production and its lipogenic effects in women of reproductive age. This finding may therefore contribute to further understanding of the mechanism of ASP regulation regarding fat storage and distribution in women.

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A STUDY OF DETERIORATION OF PULMONARY FUNCTION PARAMETERS AMONG SMOKERS AND RECOVERY AMONG EX-SMOKERS IN BUS DEPOT WORKERS

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Abstract : Smoking has deleterious effects on Pulmonary Function Test (PFT) parameters; however, evidences about recovery in ex-smokers are ambiguous. Therefore present study was conducted to quantify relative deterioration of PFT parameters and to assess reversibility of the same. A cross-sectional study was conducted on 84 bus-depot workers consisting of equal number of smokers, ex-smokers and non-smokers. PFT observations were obtained using Medspiror following standard methods and precautions. Comparisons among three groups were performed employing one-way ANOVA and post-hoc tests. There were substantial effects of smoking on PFT parameters (deterioration was up-to half). Partial recovery was found in all the parameters of ex-smokers. Frequency and duration of smoking were negatively correlated with some of the parameters. In conclusion, present study has demonstrated considerable deterioration of PFT parameters in smokers and indications of recovery in ex-smokers. Further detailed study with larger sample size and stricter definition of ex-smokers is recommended.

Key words : PFT parameters, smoking, reversal of effect, depot workers, Mysore

INTRODUCTION

Smoking has extensive deleterious effects on respiratory functions and is clearly implicated in the etiology of a number of respiratory diseases. More than 2000 potentially noxious constituents have been identified in tobacco smoke, many of which are potential carcinogens (1). Chronic bronchitis, so common among habitual smokers is not as trivial as it was thought. When it persists for years, it may progress to Chronic Obstructive Pulmonary Disorder (COPD), Corpulmonale and Metaplasia of respiratory epithelium, providing a rich soil for cancerous transformations. Epidemiological and clinical observations establish a positive relationship between smoking and lung cancer, which is overwhelming (2).

Smoking is a major risk factor for

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developing COPD. Early diagnosis of COPD is crucial to decrease the rates of morbidity & mortality. It is an established fact that smoking causes inflammation of the air ways & impairment of the lung functions. However the quantum of deterioration in different Pulmonary Function Test (PFT) parameters has great scope for variation. One of the factors that may influence the impact of smoking on the PFT parameters is their occupational exposure. Another question that is, yet to be answered is, whether the PFT parameters return back to normal once a smoker quits smoking. There are only a few studies to state if the negative effect of smoking remains even after a smoker quits this habit. Evidences about the PFT parameters returning back to normal in ex-smokers are ambiguous. Therefore the objectives of the present study were (a) to quantify the relative deterioration of various PFT parameters due to smoking, (b) to study whether the impact of smoking on PFT parameters is reversible, and (c) to assess the correlation present if any, between the various exposure (smoking) and outcome (PFT) parameters.

MATERIALS AND METHODS

It was a cross-sectional comparative study of PFT parameters in three groups viz, smokers, ex-smokers and non-smokers. First group included the subjects who were currently smoking and have smoked at least 5 pack-years. Second group included those who had smoked at least 5 pack years in the past and have quit smoking minimum one year before this study (3). Third group consisted of those who did not smoke at all.

Age and sex are almost universal predictors of any biological phenomena. Further, in case of PFT, occupation is an important potential confounder. Therefore, we restricted the study to male workers working in KSRTC bus depot located in Mysore, between the age group of 30-50 years. The study was approved by the Institutional Ethical Committee of JSS Medical College, Mysore.

Sample size and sampling

The estimation of sample size was based on determination of a difference of 10% in the mean value of different PFT parameters at 5% level of significance and 90% power. Using the mean and standard deviation (SD) for normal controls provided by Mehrotra et al (4), the required sample size was estimated to be at least 28 in each group. As the depot workers are mobile, it was very difficult to prepare a sampling frame and perform simple random sampling; we selected the subjects in the three groups according to convenience sampling.

Inclusion criteria

For smokers	: Current smokers in the age group of 30-50 years who have smoked at least 5 pack years
For Ex smokers	: Persons in the same age group who had smoked atleast 5 pack years and had quit smoking minimum one year before the study.
For non smokers	: Bus depot workers between 30-50 years who had not smoked at all.

All the three groups were exposed to the same environment.

Exclusion Criteria : For all the three groups:
Subjects with respiratory and cardiac illness
Occasional smokers

Collection of data

We visited the depot and considered the workers available. The purpose, procedure and importance of the study were thoroughly explained to the workers and their informed consent was obtained. Data on age, sex, smoking status and the history of respiratory or cardiac illness was collected to decide the eligibility of the subjects for inclusion in one of the three groups. Subsequently, the data on height, weight, frequency and duration of smoking and PFT parameters were recorded. Body Mass Index (BMI) was derived by dividing the weight in kgs by the square of height in meters. Smoking history was calculated in pack-years as the product of tobacco use in (years) and the average number of cigarettes smoked per day and dividing the product by 20 (years x cigarettes per day/20) (5).

PFT observations

It was performed with the help of Medspiror, an electronic PFT machine which is a type of flow sensing spirometer (6). Standard methods and precautions outlined in American Thoracic Society (ATS) 1994 update were followed. The parameters of PFT studied, included Forced Vital Capacity (FVC), Forced Expiratory Volume in first second (FEV_1), Forced Expiratory Flow ($FEF_{25-75\%}$) and Peak Expiratory Flow Rate (PEFR), and Maximal Voluntary Ventilation (MVV). The respiratory maneuvers were demonstrated to each subject before the test. Three reproducible tests were carried out for each measurement & the best result was selected for statistical analysis.

Statistical analysis

We estimated the mean and standard deviation to assess the level of various PFT parameters in the 3 groups and subsequently the relative deterioration among smokers and recovery among quitters was worked out in percentages. In order to compare the level of pulmonary function parameters in the 3 groups (smokers, ex-smokers and non smokers), one way analysis of variance (ANOVA) was applied at 5% level of significance followed by post-hoc tests in case of significant ANOVA test. To assess the linear relationship between different exposure and outcome parameters, Pearson's correlation coefficients were worked out and their statistical significance was tested using t-test. Data entry and statistical analyses were performed using MS-Excel and Epi-Info package respectively.

RESULTS

Basic characteristics of the subjects (Table I)

In order to assess the comparability of three groups, we compared basic parameters, namely, age, height and weight, with main focus on age which is an independent predictor in any biological phenomena. Average age of the subjects in the three groups was 43-48 years with no statistically significant difference among the groups. Similarly average height (163-166 cms) did not depict any significant difference among three groups. In case of weight, there was significant difference between smokers and

non-smokers. However, ex-smokers did not differ significantly from any of other two groups. Consequently, BMI of non-smokers was higher than that of smokers but not significantly different for ex-smokers.

Effect of smoking on PFT parameters (Table II)

Comparison of various PFT parameters under study along with the results of ANOVA and post-hoc tests has been presented in Table II. There appeared to be quite a substantial and statistically significant effect of smoking on the pulmonary functions. All the parameters were found to be deteriorated among smokers compared to non-smokers with statistically significant differences ($p<0.001$). The deterioration of the parameters due to smoking varied from a low of 22% in case of MVV to a high of 47% in case of FVC.

Recovery among ex-smokers (Table II)

It was found that among ex-smokers, there was recovery in all the parameters. However, in case of $FEF_{25-75\%}$ and MVV, the differences between current smokers and ex-smokers were not statistically significant. Regarding quantum of recovery, it was highest in case of FVC followed by FEV_1, $FEF_{25-75\%}$ and PEFR.

Correlation between different exposure and outcome parameters (Table III)

We studied the linear relationship between different measures of exposure, namely, duration, frequency and pack-years of smoking and the measures of outcome

TABLE I: Mean and Standard Deviation (SD) of various anthropometric parameters among the three groups (each 28) along with the results of ANOVA and post-hoc tests

Parameters	*Smokers*	*Ex-smokers*	*Non-smokers*	*p-value*
	Mean±SD	*Mean±SD*	*Mean±SD*	
Age (years)	44.6±8.7	48.3±8	43.3±7.7	0.068
Height (cms)	163.1±5.7	166±8.7	163.8±4.1	0.227
Weight (kgs)	63.3±10.0	64.8±10.0	70±9.0*	0.029
BMI (kg/m^2)	23.8±3.5	23.5±3.4	26.1±3.4*#	0.010

*Significantly different from Smokers ($p<0.05$); #Significantly different from Ex-smokers ($p<0.05$).

TABLE II: Mean and Standard Deviation (SD) of various PFT parameters among the three groups (each 28) along with the results of ANOVA and post-hoc tests.

Parameters	*Smokers*	*Ex-smokers*	*Non-smokers*	*p-value*
	Mean±SD	*Mean±SD*	*Mean±SD*	
FVC (ltrs)	1.91±0.606	2.99±0.744*	3.63±0.585*#	0.000
FEV_1 (ltrs)	1.74±0.518	2.72±0.735*	3.18±0.668*#	0.000
$FEF_{25-75\%}$ (ltrs/min)	2.75±0.908	3.31±1.188	4.17±1.044*#	0.000
MVV (ltrs/min)	91.68±21.09	90.11±25.31	118.1±21.96*#	0.000
PEFR (ltrs/min)	5.44±1.411	6.44±1.400*	7.58±1.574*#	0.000

*Significantly different from Smokers ($p<0.05$); #Significantly different from Ex-smokers ($p<0.05$).

TABLE III : Pearson's correlation coefficient (r) between different exposure and outcome parameters under study along with p-values for the statistical significance.

Exposure parameters	*Outcome parameters*	*Smokers*		*Ex-Smokers*	
		r	*p-value*	*r*	*p-value*
Frequency	FVC	0.193	0.326	–0.16	0.417
	FEV_1	0.263	0.176	–0.188	0.338
	$FEF_{25-75\%}$	0.374	0.050	–0.471	0.011
	MVV	0.091	0.645	–0.456	0.015
	PEFR	0.078	0.693	–0.274	0.159
Duration	FVC	–0.104	0.597	–0.412	0.029
	FEV_1	–0.052	0.791	–0.352	0.066
	$FEF_{25-75\%}$	–0.042	0.832	–0.058	0.770
	MVV	–0.271	0.163	–0.021	0.916
	PEFR	0.158	0.423	–0.208	0.288
Pack years	FVC	0.123	0.532	–0.507	0.006
	FEV_1	0.221	0.258	–0.469	0.012
	$FEF_{25-75\%}$	0.322	0.095	–0.466	0.013
	MVV	–0.219	0.263	–0.336	0.080
	PEFR	0.348	0.070	–0.401	0.034

consisting of PFT parameters. No significant correlation was observed among current smokers. However, many statistically significant correlation coefficients were noticed among ex-smokers. Frequency of smoking was significantly correlated with $FEF_{25-75\%}$ and MVV. Duration of smoking was significantly correlated with FVC. Pack-years of smoking which is a combination of other two exposure variables, was significantly correlated with most of the PFT parameters. All the significant correlations were negative indicating that higher the exposure of smoking, lower is the value of PFT parameter.

DISCUSSION

Respiratory disorders develop much earlier and therefore respiratory morbidity is also higher in smokers (7). It is a known fact that pulmonary function values are influenced by many socio-demographic, physiological and environmental factors like race, age, sex, height, weight & other unknown variables having a wide range of normal values (8). However, these exposures are by and large irreversible and therefore the risk is difficult to avoid. On the other hand, the role of smoking in the impairment of ventilatory functions is also an established fact but the same is potentially reversible. Hence this study was conducted with the main emphasis on the assessment of reversibility of the deterioration in PFT parameters.

Three groups under study were comparable on gender, occupational exposure and basic physiological parameters except that average weight and BMI were higher among non-smokers compared to other groups. We also know that if there is any correlation between BMI and PFT parameters, it is negative. Thus higher BMI

may result in reduction in the values and therefore significant differences observed may not be explained by the differences in BMI.

This study has substantiated the findings of other studies (9-11) that tobacco smoking has an impact on all the PFT parameters and the resultant deterioration may be due to the carbon monoxide, tar and other toxic contents of tobacco smoke adversely affecting the alveoli (12). The lower values of FVC, FEV_1, and $FEF_{25-75\%}$ in smokers when compared with non smokers were also observed in a study conducted on workers exposed to dust and fumes (7). The statistically significant reduction in values of FVC suggests that smoking is an initial step contributing to the development of COPD by causing narrowing of the airways. Reduction in $FEF_{25-75\%}$ values can be used as a screening measure to detect airway narrowing. $FEF_{25-75\%}$ values are known to decrease in early COPD (13) which is demonstrated in our study also. Analysis of relation between smoking state and ventilatory functions disclosed significant reduction of PEFR in current smokers .Some studies have reported a relationship between narrowing of small airways and a decrease in FEV_1, which was observed in our study also.

Smoking is associated with abnormalities of airway structure (11). The significant correlation of MVV with the frequency of smoking indicates the proportionate degree of weakness of respiratory muscles and reduction in respiratory reserve in ex smokers. This is similar to the observation made by an ICMR study which states that there is a reduction in MVV in direct proportion to the degree of weakness of respiratory muscles in malnourished patients (14).

Though some studies have suggested that ex smokers still show lung damage and the negative effect remains even after a smoker quits (15), our study revealed recovery in all the pulmonary functions in ex smokers. But still these lung functions were significantly below that of the non smoking group. These findings are similar to another study where ex smokers had better lung function values than smokers, but their mean curves were below the values of non smokers (9). Another study conducted on elderly men had also reported recovery of PFT parameters in ex-smokers (16).

Recovery however depends on many factors, such as, duration, frequency and type of smoking before quitting and the resultant deterioration, duration since quitting and many other life style related factors. It was not possible to consider these factors in the present study given the sample size which was estimated to be enough to detect the difference in different groups. Therefore a further study with the larger sample size incorporating associated factors may be recommended. It may be noted that behavior of tobacco use is a habit very difficult to change, even with medicinal aids for cessation. Only a small proportion of smokers stop smoking successfully on their own. Therefore, once established, the facts about recovery after quitting the habit may be important information useful for counseling the smokers for quitting the habit.

Conservatism is an important issue in any study related to smoking. Generally the smokers are expected to be conservative in

reporting the duration and frequency of smoking. This is more so in case of ex-smokers. There is a possibility that a person claiming to have quit the habit, smokes occasionally. This might have had an impact on the findings about the reversibility of the deterioration in the PFT parameters. Therefore the estimate of quantum of reversibility reported by present study may be the conservative one. More stringent criteria in selecting ex-smokers may resolve the issue of conservatism in reporting.

In conclusion, this study has demonstrated the substantial deterioration of pulmonary functions in smokers and the indications of recovery among ex-smokers. However, in view of the limitations and potential biases, there is a possibility of conservatism in the estimates of deterioration and recovery. Therefore, we recommend further active research with larger sample size taking into account the quantum of exposure and duration of quitting. This may possibly establish the recovery of PFT parameters among ex-smokers, a very important fact from public health policy point of view.

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EFFECT OF YOGA NIDRA ON PHYSIOLOGICAL VARIABLES IN PATIENTS OF MENSTRUAL DISTURBANCES OF REPRODUCTIVE AGE GROUP

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Abstract : Aim of this study was to see any effect on autonomic functions in menstrual disturbances patients after Yoga Nidra practice. The subjects for the study were 150 females with menstrual irregularities, 28.08±7.43 years of mean age, referred from department of Obstetrics and Gynecology CSMMU, UP, Lucknow. Subjects were divided randomly in to two groups' intervention and in control groups -seventy five (75) in each group. Out of these, one hundred twenty six (126) completed the study protocol. The yogic intervention consisted of 35-40 minutes/day, five days in a week till six months. An autonomic function testing was done in both the groups at zero time and after six months. A significant positive effect was observed when yoga therapy was used as an adjunct in the patients of menstrual disturbances. There were significant improvements in the blood pressure, postural hypotension and sustained hand grip, heart rate expiration inspiration ratio and 30:15 beat ratios of the subjects after yogic practice.

Key words : yoga nidra, menstruation, sympathetic, parasympathetic

INTRODUCTION

The human body is a self-regulating mechanism that is constantly adjusting itself in tune with its own needs and capacities (1) The menstrual cycle is a sequence of events that occurs once in a month in a sexually mature female (2). Menstrual disorders have become widespread over the last few generations so that menstrual difficulties cause, as much wretchedness as the common cold and medical insight into this problem is equally limited. The menstrual disorders are known to have cause-effect relationship with hormonal and pathophysiological status of body. As part of many adolescence changes, experiences and challenges, menstruation onset, menarche, is a very significant event. Dysmenorrhea, or menstrual pain, is defined as chronic pelvic pain that occurs in about 15% to 70% of young women (3-4). Wood et

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al. (5) found that dysmenorrhea is most common between the ages of 15 and 19 and that 82% of the women in this age group experience such pain. This pain gradually increases from the age of 15 and then begins to decline by the age of 20 and following parity (6, 7).

This study was designed to verify if the yoga nidra intervention is helpful in providing balance between sympathetic and parasympathetic nervous system in the patients of menstrual disorders. The nervous system is the cornerstone ability to perceive, to adapt, and to interact with the world around. It is the means by which persons receive, process and then respond to messages from the environment and from inside their body. Sympathetic and parasympathetic divisions work in synergy and complement each other better when yogic practices are used. Elevated resting heart rate is a risk factor for sudden cardiac death, even in the general population (8-10).

METHODS

Subjects with menstrual irregularities visiting the Department of Gynecology, CSMMU were included in the study. A senior gynecologist referred them after being examined for their physical health and medication status. Medication (tranexemic acid, ethamsylate, madroxy progesterone, norethisterone Ethinyl estradiol, Levonorgestrel) was provided to both the groups. The institutional Research Ethics-Committee approved this study. After signed informed consent by the subjects anthropometrics measurements were taken. Each subject was randomly assigned to one of the two groups: (i) Medication + yoga Nidra, (ii) Medication, using a random number generator such that equal numbers were recruited into each group. A statistician not associated with this study generated the randomization scheme with block of size four for up to 196 patients. These numbers were pasted on identical opaque envelopes containing yoga and non-yoga. The numbers were noted for group 1 (yoga Nidra) and group 2 (non yoga) and sealed in a big envelope. After randomization patients in Intervention and control groups were (n=75). Yoga Nidra sessions were guided by well-educated and trained yoga instructors selected by an expert committee. Most of the participants were from local population. Yoga Nidra sessions were free of cost and all the necessary facilities were provided to the participants like airy room, yoga mats etc. Subjects were regularly motivated for Yoga Nidra practice. Therefore the flow of patients was continued during study. Patients, who dropped the study, also did not differ significantly in terms of age. Before study all the subjects were asked to maintain their routine activities and not initiate any new physical activities for that duration. Autonomic function testing was done with the help of Cardiac Neuropathy Analyzer (CANwin) and Variowin, Banglooru in both the groups at the beginning and after six month of the study.

Autonomic functions tests

A battery of six standard tests was used to assess the integrity of autonomic function status of the subjects (11). These tests were helpful in assessment of reactivity of divisions of ANS, the sympathetic and parasympathetic. Autonomic function tests that were performed for parasympathetic and sympathetic functions. Tests for sympathetic

reactivity - Blood pressure Response to lying to standing test and handgrip Test (HGT) (12, 13).

Heart rate response to standing from the supine posture (30:15 ratio)

$$30^{th}:15^{th}\ \text{ratio} = \frac{\text{Maximum R - R interval around } 30^{th}\text{ beat}}{\text{Minimum R - R interval around } 15^{th}\text{ beat}}$$

Slow deep breathing test :

$$\text{E : I ratio} = \frac{\text{Average maximum R - R interval during expiration}}{\text{Average minimum R - R interval during inspiration}}$$

Valsalva maneuver (14) :

$$\text{Valsalva ratio} = \frac{\text{Longest R - R interval after maneuver (phase IV)}}{\text{Shortest R - R interval during maneuver (phase II)}}$$

Heart Rate Variability (HRV)

Heart rate variability (HRV) is one of the most widely used methods for measuring cardiac autonomic activity in humans (15). HRV reflects the balance between the sympathetic and parasympathetic regulatory control of the heartbeat; low HRV suggests excessive cardiac sympathetic modulation, inadequate cardiac parasympathetic modulation, or both (15).

Sample size for this study was 120, when type I error 0.05 and power was 80%, expected difference in population means was 0.71, standard deviation within group 1.95 and ratio between intervention and control group was 1, calculated with the help of Power and sample size calculator, Version 2.1.30 (William 2003). After six months 126 subjects completed study protocol, intervention group (n=65) and control group (n=61). Patients, who dropped the study, also did not differ significantly in terms of age.

The patients of menstrual disorders were diagnosed by the following diagnostic criteria after taking detailed clinical history – Amenorrhea, dysmenorrhea, Oligomenorrhea, Polymenorrhea, Menorrhagia, Metrorrhagia, Menometrorrhagia and Hypomenorrhoea were included for the study and women having known gynecological neoplastic diseases requiring surgery, Pelvic inflammatory disease (PID) or Pregnancy were excluded from the study. Subjects who did not participate in yogic intervention classes (>80% Yoga Nidra classes) were also excluded from the study.

Anthropometric measurements were taken before and after study. Height was measured with the participants standing without shoes and was recorded to the nearest of 0.5 cm. Weight was measured using a digital scale, with the participants wearing light clothing, and was recorded to the nearest 100 grams.

Under the guidance and supervision of yoga experts and faculty, subjects performed Yoga Nidra practice. Yoga Nidra, which is derived from the tantras, is a systematic method of inducing complete physical, mental and emotional relaxation (16). Yoga Nidra is performed in shavasana. It has several steps like – Resolve, Rotation of consciousness, and awareness of the breath, Feeling and sensation, Visualization, ending the practice with resolve (17). It helps in restoring mental, emotional, and physical health by way of relaxation, and makes the mind more conducive to *pratyahara* – withdrawing senses from their objects, *dharana* - concentration, and meditation. Such a practice helps harmonize two hemispheres of the brain and the two aspects

of autonomic nervous system (sympathetic and parasympathetic). The impressions in the subconscious are brought to surface, experienced and removed. Thus, the fixation of awareness on the body is replaced with the awareness linked to subtler aspects of prana (the life force) and spiritual dimensions allowing for maximizing of the pure yet unmanifested potential within. Total duration of this practice was 35-40 minutes/ day, five days in a week in the morning for six months. Yoga Nidra practice was done in the department of physiology CSMMU UP, Lucknow.

Statistical analysis

The two groups were compared on these scores using one-way ANOVA. P value of <0.05 was taken to be significant. InStatS software version 3.05 was used for the analysis (www.graphpad.com).

RESULTS

One hundred and seventy four women attended the pre-screening visit, but 24 were not participating due to family problems. A total of 150 women were randomly assigned to intervention and control group. Data for analysis is available for 126 women; 24 women were lost to analysis for several reasons. Tables I show the baseline characteristics for total patients intervention and control group. Relief in symptoms after six months in both groups are given blow in table 2 and Q-statistic (IS) for testing homogeneity of risk differences have been applied.

TABLE I: Baseline demographic profile of the subjects participated in the yogic intervention program.

Variables	*Control group (N=75)*	*Intervention group (N=75)*	*P value*
Age (yrs)	27.62±7.78	28.53±7.07	0.46
BMI (kg/m^2)	21.98±4.22	23.21±4.88	0.10
WC (cm)	75.61±12.92	77.36±13.10	041
HC (cm)	90.12±11.93	94.52±12.19	0.02
WHR	0.83±0.07	0.81±0.06	0.05

Data presented are mean±SD, BMI, Body Mass Index; WC, Waist Circumference, HC, Hip Circumference, WHR, Waist Hip Ratio.

DISCUSSION

Individuals recruited for this intervention program constitute a representative sample of the large number of patients suffering from menstrual problems. Patients

TABLE II: Relief in symptoms after six months in both Intervention and Control Groups.

Variables	*Control group*		*Intervention group*			
	Pre (n=75)	*Post (n=61)*	*Pre (N=75)*	*Post (n=65)*	*Q-static,*	*P Value*
Pathological Amenorrhea	30	23	32	18	1.20;	0.27
Dysmenorrhea	32	26	34	24	0.49;	0.48
Oligomenorrhea	21	14	18	6	1.02;	0.31
Polymenorrhea	14	6	16	5	0.34;	0.56
Menorrhagia	11	8	13	9	0.07;	0.78
Metrorrhagia	17	14	16	9	0.65;	0.42
Hypomenorrhea	10	4	13	8	0.05;	0.82

TABLE III : Variables in control and intervention groups before and after the study.

Variables	*Control group*		*Intervention group*		*P value*
	Baseline (N=75)	*After six months (N=61)*	*Baseline (N=75)*	*After six months (N=65)*	
BMI (kg/m^2)	21.98±4.23	21.12±4.08	23.21±4.89	22.24±4.85	0.06
SBP (mmHg)	122.86±7.24	121.21±7.39	121.96±7.63	118.98±7.03*	0.01
DBP (mmHg)	78.84±8.41	75.37±7.97	77.37±8.63	73.15±8.08**,f	0.0005
Heart Rate (bpm)	71.72±8.81	70.67±8.45	72.07±9.71	69.14±9.14#,f	0.01
Postural Hypotension (mm Hg)	8.76±2.45	7.05±1.94**	8.97±3.03	7.2±2.07**,#,f	0.0001
Sustained hand grip (mm Hg)	13.91±2.41	12.55±2.26*	14.52±2.58##	13.44±2.67	0.0001
Expiration-Inspiration Ratio	1.36±0.33	1.25±0.31	1.29±0.36	1.19±0.37*	0.03
30:15 Beat Ratio	1.35±0.38	1.19±0.33	1.33±0.44	1.16±0.41*	0.01
Valsalva Ratio	1.77±0.68	1.59±0.52	1.81±0.71	1.68±0.55	0.17
LF	1374.8±2058.9	1367.8±2163.5	1314.9±1981.0	1303.2±2025.7	0.99
HF	891.0±1359.8	818.2±939.9	848.3±1327.6	824.9±1337.0	0.98
LF/HF	1.47±0.67	1.35±0.43	1.56±1.03	1.50±0.70	0.43

Data presented are mean±SD. Analysis of data was done by one-way ANOVA and post-hoc by Tuky-Krammer test. The * depicts comparison with Control – Baseline and the # depicts comparison with Control – after 6 months, and the f depicts comparison with Study-Baseline. ***P<0.001; ###P<0.001; fP<0.05.

participating in the intervention group were requested to follow yoga nidra intervention program as prescribed by the researcher. In the light of above tables it is clear that yoga nidra practices are helpful to prevent the menstrual problems of reproductive age group women. The practice of yoga generally includes meditation, relaxation (yoga nidra), breathing exercises and various physical postures (19). In addition, adults participating in a yoga intervention found that yoga was easily learned and performed (20). Studies conducted in different region showed that both, activity scheduling and relaxation training were effective treatments for spasmodic dysmenorrhea, with both treatments producing improvements on general measures of dysmenorrhea, a symptom severity measure, and an activity measure (21).

In present study SBP, DBP, postural hypotension and sustained hand grip, in Table III changed significantly after yoga nidra practice, our results corroborate with the findings of Datey et. al. found that yoga nidra therapy adopted either alone or as an adjunct therapy has been observed to reduce systolic readings (SBP) by an average of 15-20 mm Hg, and diastolic readings (DBP) by 10 mm Hg after 3 weeks or more practice (22-24). A recent, study conducted at the Stanford University School of Medicine (USA) demonstrated that the drop in blood pressure induced by daily yoga nidra practice has a far-reaching effect, extending throughout the day, and is not merely a transient effect coincident with the practice session (25). Another controlled study, which was conducted at the Langley Porter Neuropsychiatry Institute in California, found that reduction in blood pressure and anxiety levels in hypertensive patients continued for 12 months after yoga nidra training (26). In the Table III, heart rate, expiration inspiration ratio and 30:15 beat ratios changed significantly after yoga nidra practice in this study. Telles et. al. also reported that the rate of respiration (RR) and heart rate (HR) decreased significantly (27). But there was

no significant change observed in other ANS variables. Yogic practices help to distract our attention from chronic worrying, giving us a respite from daily distress or current problems all of which help to reduce heart rate which is also seen in the present study.

All the parameters given in the table 2 clinically changed but not statistically significant. Yet it was observed that after Yoga Nidra practice patients relieved from painful cramps, heavy bleeding and irregular periods. Although number of patients–suffering, reduced in intervention and control groups because both groups were taking medication, reduction in intervention was higher than control group. How yoga nidra works it is not fully documented but it has been reported previously that yoga nidra is deep relaxation technique. The relaxation response is characterized by a decrease in the activity of the sympathetic nervous system that results from conditioning and training. During this response there is a decrease in the oxygen consumption, heart rate, blood pressure and respiration rate and an increase in the alpha waves of the electroencephalogram. The relaxation response is not just simple relaxation. In simple relaxation, changes in the rate of respiration, oxygen consumption and alpha wave activity do not occur. The relaxation response is thought to modify the way in which stressful stimuli affect the sympathetic nervous system.

Menstrual disorders such as amenorrhea/ oligomenorrhea depend on many factors, including race, genetic makeup, BMI, and family history. Most of the studies on menstrual disorders have emphasized mainly on the drug management, while only a few stressed on alternative practices and the perceptions in different settings. It is well known that every health problem not only presents it with different epidemiological profiles in different population settings but also perceived and managed differently. It is not yet certain that altered ANS activity is responsible for the increased risk of mortality and medical morbidity associated with menstrual disorder patients. Unfortunately such types of study have not been performed in menstrual disorder patients.

Limitations of this study were that we have included Amenorrhea, Dysmenorrhea, Oligomenorrhea, Polymenorrhea, Menorrhagia, Metrorrhagia, and Hypomenorrhoea in the present study together. Sample size is not enough to analyze oligomenorrhea and menorrhagia separately. The phase of the menstrual cycle was based on the participants reports not on ultrasound scans this is also a limitation of the study.

In conclusion the present study demonstrated the efficacy of Yoga Nidra on autonomic nervous system variables in patients of menstrual irregularities. Yoga Nidra practice is helpful in patients of menstrual disorders.

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EFFECTS OF CURCUMIN ON THE GASTRIC EMPTYING OF ALBINO RATS

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Abstract : Curcumin (diferuloylmethane), a polyphenol, is an active principle of the perennial herb Curcuma longa commonly known as turmeric. Turmeric (CURCUMA LONGA L.) is a medicinal plant extensively used in Ayurveda, Unani, and Siddha medicine as a home remedy for various diseases including biliary diseases, cough, hepatic diseases, wound healing. However studies on the effect of curcumin on the gastric emptying are nearly nonexistent. It is hypothesized that curcumin may have an effect on gastric emptying. For this reason the present study was aimed to study the effect of curcumin on gastric emptying. Rats were divided into 5 groups (Group I – Group V), based on the time interval between administration of curcumin/vehicular fluid to administration of barium sulphate (Group I – 1 hr, Group II – 8 hrs, Group III – 16 hrs, Group IV – 24 hrs, Group V – 48 hrs). Each group was further divided into two sub-groups, Group A (control) and Group B (experimental), containing 6 rats each. Rats in experimental group were administered curcumin intragastrically, in the dose of 1 gm/kg body weight, suspended in normal saline (0.9% NaCl). The controls were given vehicular fluid intragastrically, in volume equal to the experimental animals. It was observed that there was a decrease in the gastric emptying in all the experimental groups.

Key words : gastric emptying curcumin barium sulphate

INTRODUCTION

Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern. The Indian system of holistic medicine known as Ayurveda uses mainly plant-based drugs or formulations to treat various ailments

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including cancer. Of the approximately 877 small molecule drugs introduced worldwide between 1981 and 2002, most (61%) can be traced back to their origins in natural products (1-4).

Curcumin (diferuloylmethane), a polyphenol, is an active principle of the perennial herb Curcuma longa commonly known as turmeric. TURMERIC *(CURCUMA LONGA L.)* is a medicinal plant extensively used in Ayurveda, Unani, and Siddha medicine as a home remedy for various diseases. For that reason a number of references to the plant are found in classical ayurvedic text such as Charaka Samhita, Sushuruta Samhita, Ashtanga Hridya and Sharangdhar Samhita. These texts described the use of turmeric for a number of disorders pertaining to many systems including abdominal pain, joint pain, swelling and snake bite etc (5-7).

Studies, in the human being and in the experimental animals have shown the beneficial effect of curcumin on the function of gastrointestinal tract. It increases bile secretion in anesthetized dogs and rats. It elevates the activity of pancreatic lipase, amylase, trypsin and chymotropism. Sodium curcuminate inhibit castor oil induced diarrhoea suggesting action of drug on the smooth muscle cells of gastrointestinal tract (8-12). However studies on the effect of curcumin on the gastric emptying are nearly nonexistent. It is hypothesized that curcumin may have an effect on gastric emptying. For this reason the present study was aimed to study the effect of curcumin on gastric emptying.

MATERIALS AND METHODS

Experimental animals

Albino rats of wistar strain, weighing 130-170 gm, of either sex, raised under standard laboratory conditions were obtained from Indian Veterinary research institute, Izat Nagar, Barellie, Uttar Pradesh. The animals were housed in polycarbonate cages of size 35 cm × 23 cm × 16 cm. Four rats per cage were kept. The animals were fed cooked food ad libitum with free access to water. All experiments in rats were carried out in accordance with the recommendation of guidelines for care and use of laboratory animals approved by Institutional Animal Ethics Committee.

Drugs curcumin

Curcumin was obtained in the form of capsule containing 500 mg of curcumin from INDSAFF, Batala. Curcumin (diferuloylmethane), a polyphenol, is an active principle of the perennial herb Curcuma longa commonly known as turmeric. The yellow-pigmented fraction of turmeric contains curcuminoids, which are chemically related to its principal ingredient, curcumin. The major curcuminoids present in turmeric are demethoxycurcumin (curcumin II), bisdemethoxy-curcumin (curcumin III), and the recently identified cyclocurcumin. The major components of Commercial curcumin are curcumin I (77%), curcumin II (17%), and curcumin III (3%) (1).

Dosage

Dose of curcumin was calculated as per

1 gm/kg body weight (13). The capsule containing 500 mg of curcumin was dissolve in normal saline (0.9% NaCl) to make 5 ml suspension of the drug, so that each ml of the suspension consist of 100 mg of curcumin.

Barium sulphate

Barium sulphate (Trade name- microbar-HD manufactured by Eskay Fine chemicals) purchase from medical store.

Weighing scale

Electronic weighing scale ACE-1000 was used to weigh the gastric content. This scale can measure up to 0.1 gm accurately.

Hot air oven

Used to dry up the washed out gastric content at 70°C–75°C for 8 hours.

Acute toxicity study

Six rats were taken for acute toxic effect of curcumin. The animals were fasted overnight and the curcumin was administered intragastric in the dose of 2 gm/kg body weight. Animals were observed continuously for first 3 hrs and were monitored for three days for mortality and general behavior of animals, signs of discomfort and nervous manifestations. No mortality and adverse effects were observed with this dose.

Gastric emptying study

Sixty rats were divided into 5 groups (Group I – Group V), based on the time interval between administration of curcumin/ vehicular fluid to administration of barium sulphate (Group I – 1 hr, Group II – 8 hrs, Group III – 16 hrs, Group IV – 24 hrs, Group V – 48 hrs). Each group was further divided into two sub-groups, Group A (control) and Group B (experimental), containing 6 rats each. Rats in Group B were administered curcumin intragastrically by the naso-gastric tube reaching up to the lower 1/3rd of esophagus, in the dose of 1 gm/kg body weight, suspended in normal saline while rats in Group A were given vehicular fluid (0.9% NaCl) in equal volume as that of curcumin suspension given to experimental group.

After requisite time as per Group I – Group V, in both, Group A and Group B, rats were administered 4 ml of barium sulphate suspension containing 3.2 gm of barium sulphate in isotonic saline, through a naso-gastric tube reaching up to lower third of the esophagus. 30 min after barium sulphate administration the animals were sacrificed by cervical dislocation. Abdomen was opened by midline incision and ligatures were applied at the oesophagogastric junction and gastroduodenal junction. The stomach was striped out carefully.

The stomach was cut open and washed for its luminal contents into a beaker with normal saline (0.9% w/v) till no barium particles can be visualized under mucosal surface. The stomach washing was centrifuged at 3000 rpm for 5 min in a clinical centrifuge. The sediments were dried in hot air oven (70°C–75°C) for 8 hours. The total weight of luminal contents was measured with electronic balance.

Statistical analysis

Mean and standard error of all the observations were calculated and comparisons were done between experimental and control groups by applying Student's t test (unpaired). Comparisons of the effect of curcumin on the gastric emptying among different experimental groups were done applying one way ANOVA.

RESULTS

After the intra-gastric administration of single dose of curcumin, there was decrease in gastric emptying in all the experimental groups as compared to control groups. On applying Student's t test, decrease in gastric emptying in Group I to Group III was statistically significant, while in Group IV and Group V it was statistically insignificant as compared to control groups. On applying one way ANOVA between different experimental groups, there was statistically significant decrease in gastric emptying in Group I as compared to other Groups. While comparing Group II and Group III with Group IV and Group V, there was statistically significant decrease in gastric emptying as compared to Group V. However the difference among Group IV and Group V was statistically insignificant (Table I).

TABLE I: Comparisons of effect of curcumin on gastric residue (Mean±SE) following intragastric administration of single dose of curcumin (1 gm/kg body wt) in different groups.

Groups	*% portion of barium sulphate recorded in stomach*		*P value*
	Control (n=6)	*Experiment (n=6)*	
Group I	23.60±3,837	59.38±3.608	<0.0001
Group II	24.14±3.754	46.88±2.282$^{\#\#}$	0.0005
Group III	23.83±3.894	40.63±1.398$^{\#\#\#}$	0.0019
Group IV	23.44±3.904	31.25±1.745$^{\#\#\#}$	0.0976
Group V	23.72±4.394	23.44±2.387$^{\#\#\#,***,\wedge\wedge\wedge}$	0.956

Comparison of control with experimental group was done by *t* test and inter-groups (I-V) comparison among experimental group was done by one-way ANOVA. # mark represents comparison with group I, * mark represents comparison with group II and ^ mark represents comparison with group III $^{\wedge,*,\#}$<0.05; $^{\wedge\wedge,**,\#\#}$<0.01; $^{\wedge\wedge\wedge,***,\#\#\#}$<0.001.

DISCUSSION

The results of the present study revealed that there was delay in gastric emptying following intragastric administration of curcumin. Limited information exists about the mechanism by which curcumin delays gastric emptying. Gastric emptying is dependent on the organization of motor activity in the proximal stomach, antrum pylorus and duodenum (14-16). The delivery of nutrients from stomach to small intestine is closely regulated, largely as the result of feedback from chemoreceptors and mechanoreceptors (17). It has been demonstrated that vagal stimulation increases the release of nitric oxide (NO) from the gastric myenteric plexus, which mediates nonadrenergic, noncholinergic (NANC) relaxations and accommodation reflex of the stomach (18, 19). NO synthase inhibitors given i.v. at doses that inhibit NO synthase, delay gastric emptying through mechanisms which are unrelated to changes in arterial blood pressure (20).

In animal studies inhibition of NO synthase is associated with suppression of proximal gastric relaxation, stimulation of antral, pyloric and duodenal motility and slowing of gastric emptying (21-26). Chan MM et al. reported that curcumin inhibits nitric oxide synthesis by inhibiting nitric oxide

synthase gene expression (27). Sreejayan N, Rao MNA reported that curcumin has nitric oxide scavenging property (28).

The delayed gastric emptying as observed in our study could be explained by the NOS inhibitory action (27) and scavenging property (28) of curcumin, however other mechanisms may also be involved. Further experimental work needs to be done to establish its mechanisms of its action.

In conclusions present study suggests that curcumin delays gastric emptying. It may be used as an adjuvant for treatment of diseases in which gastric emptying is increased such as Zollinger-Ellison syndrome.

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SHORT COMMUNICATION

IMMEDIATE EFFECT OF MUKHA BHASTRIKA (A BELLOWS TYPE PRANAYAMA) ON REACTION TIME IN MENTALLY CHALLENGED ADOLESCENTS

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Abstract : Mentally challenged individuals are known to have slower speed of reaction. As a previous study has shown immediate improvement in reaction time (RT) following *mukha bhastrika,* a bellows type of *pranayama,* we planned to study the effect of this *pranayama* in mentally challenged adolescents. 34 mentally challenged adolescents (15.1±0.806 y) studying in a school for Special Needs were recruited as they have been receiving yoga training once a week for more than 3 years. Exclusion criteria were inability to either perform *mukha bhastrika* or to understand procedure for testing RT. Visual (VRT) and auditory reaction time (ART) was measured using RT apparatus before and after nine rounds *of mukha bhastrika* and a control period of ten minutes of normal activities to rule out any test-retest practice effect. Analysis of non-intervention period values showed that the reliability in terms of reproducibility of the observation for both VRT (r=0.87) and ART (r=0.95) was excellent. *Mukha bhastrika* produced an immediate and significant decrease in both VRT and ART. There was a statistically significant decrease in VRT (P<0.0001) from 296.15 ms±13.49 to 263.59 ms±12.53 and ART (P<0.0001) from 247.88 ms±14.33 to 217.35 ms±11.36 following *mukha bhastrika.* Decrease in RT signifies improved central neuronal processing ability. This may be due to greater arousal and faster rate of information processing, improved concentration and/or ability to ignore or inhibit extraneous stimuli. *Mukha bhastrika* may be altering afferent inputs from abdominal and thoracic regions, in turn modulating activity at ascending reticular activating system and thalamo-cortical levels. It is suggested that yogic breathing techniques like *mukha bhastrika* be used as an effective means of improving neuro-muscular abilities in special children.

Key words : reaction time, mukha bhastrika, mental retardation, central processing

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INTRODUCTION

Yoga has been found to be an effective adjunct therapeutic modality in numerous health conditions and is being widely used all over the world. The diverse applications of yoga in rehabilitation of mentally and physically handicapped as well as visually impaired children have been demonstrated earlier with significant decrease in their abnormal anxiety levels (1).

Mental retardation is one of the conditions in which yoga may have great potential. Though there are some documented reports (2), very few have scientifically studied this effect and hence more studies are required to streamline the use of yoga as an adjunct therapy in such children.

Uma et al (3) have reported that yoga improved IQ in MR children and that the Binet Kamath (BK) test scores for general mental ability improved significantly in the yoga group. They suggested that the breath control obtained through yoga increases psycho-motor coordination. They concluded that yoga improves concentration, attention-span and enhances IQ and memory power by gaining conscious control over the mind.

The neurological benefits of yoga have interested scientists all over the world. It has been reported to be beneficial in both peripheral nerve function as well as central neuronal processing (4, 5, 6). One of the simple and effective methods of studying the central neuronal processing is the RT that is the interval between the onset of a signal (stimulus) and the initiation of a movement response. It is an indirect index of central neuronal processing and is a simple means of determining sensory-motor association, performance and cortical arousal. Decrease in RT indicates an improved sensory-motor performance and an enhanced processing ability of the central nervous system. It is a sensitive and reproducible test that can be measured with a simple apparatus and setup.

It has been found that changes in breathing period produced by voluntary control of inspiration are significantly correlated to changes in RT (7). Some studies on yoga have shown that regular practice of yoga over a period of a few weeks to a few months can significantly decrease VRT and ART (4, 5). Not many have however studied the acute and immediate effects of yoga techniques on RT.

A previous study from our laboratories reported a significant and immediate decrease in RT following the practice of nine rounds of mukha bhastrika, a bellows type of pranayama in normal school children (8). It has been previously reported that mentally challenged individuals show specific motor performance deficits on measures of RT, aiming, dexterity and that their motor performance measures are considerably longer compared to the non retarded (9).

Mukha bhastrika is a yogic technique in which the breath is actively blasted out in multiple 'whooshes' with forced abdominal contractions. After taking up Vajra Asana, a straight back sitting position, a deep inhalation is performed with awareness of the sequential expansion of the lungs. The mouth is then puckered up into Kaki Mudra, the crow beak gesture and the breath is blasted out in multiple, forceful expulsions

while simultaneously bringing the head down to the ground. Then, with a deep inhalation, the head is raised slowly and the subject comes back to the sting position. This constitutes one round of mukha bhastrika that is one of the practices being taught on a regular basis in all pranayama classes in the Gitananda tradition. This is also one of the techniques taught in regular yoga training imparted for special children in Pondicherry as part of the outreach programmes of ICYER and Yoganjali Natyalayam, Pondicherry, India.

Keeping all of this in mind, this study was planned to investigate the acute effects of mukha bhastrika on VRT and ART in mentally challenged children. Since the study was done on mentally challenged subjects and as we wanted to rule out potential practice effects on the readings, we included a non-intervention period and performed test-retest analysis to ensure reliability and reproducibility of the readings.

MATERIALS AND METHODS

Thirty four children (21 male, 13 female) with MR studying in SADAY School for Special Needs, Pondicherry, were recruited for this study by accidental sampling method as they have been receiving yoga training once a week for 2-3 years. Their mean age was 15.1±0.806 and mean IQ was 54.88±2.51. two of the children belonged to the severe mental retardation category (IQ 20-34), 10 to the moderate mental retardation category (IQ 35-49), 19 to the mild mental retardation category (IQ 50-69) and 3 were in the borderline intellectual functioning category (IQ 70-84) according to the International Classification of Diseases-10.

Exclusion criteria were the inability to either perform mukha bhastrika or to understand the procedure for testing RT. Of the 63 students studying in the school, only 34 of those children who could perform mukha bhastrika in the proper manner, as well as understand the procedure of testing RT were recruited for this study. Informed consent for the study was obtained from the head of the institution on behalf of the special children and ethical clearance obtained from that institution as well as ICYER.

RT apparatus manufactured by Anand Agencies, Pune, was used for the study. The instrument has a built in 4 digit chronoscope with a display accuracy of 1 ms. It features four stimuli, two response keys and a ready signal. Switches for selecting right or left response key for any stimulus is provided. In the present study simple ART was recorded for auditory beep sound stimulus and simple VRT for red light stimulus. The subjects were instructed to release the response key as soon as they perceived the stimulus. The signals were given from the front of the subjects to avoid the effect of lateralised stimulus and they used their dominant hand while responding to the signal (10). All subjects were given adequate exposure to the equipment on 2 different occasions to familiarize them with the procedure of RT measurement.

RT measurements were done before and after a non-intervention period of 10 minutes where the subjects continued their normal activities between the recordings. Test-retest study was done on these values to assess reliability and reproducibility of the observations and to rule out any changes

that could be resulting from 'practice effect'. RT was then recorded before and after the practice of nine rounds of mukha bhastrika. To avoid any extraneous influences due to the recording on different days, one half of the subjects performed non-intervention period recordings on day-1, while the other half did the mukha bhastrika recordings. This was then reversed on day-2. More than 8-10 trials were recorded and the average of the lowest three similar observations was taken as a single value for statistical analysis (10).

Data are expressed as mean±SEM. All statistical analysis was carried out using SPSS 13.0. The reliability and reproducibility of the observations of VRT and ART in the non-intervention period were assessed by using Test-Retest study using correlation analysis. The distribution of both VRT and ART was assessed by using Kolmogrov Smirnov (KS) test. The immediate effect of mukha bhastrika on RT was assessed by using Students t (paired) test. Correlation Analysis (Karl Pearson Coefficient of Correlation) was carried out to assess the test retest reliability of the observations in the non-intervention period to rule out 'practice effect". All statistical analysis was carried out at 5% level of significance and a P value <0.05 was taken to indicate significant differences between groups of data.

RESULTS

The results are given in Table I. Mean VRT score at baseline was 296.15±13.49 ms and there was no significant difference between male and female subjects though mean score of male subjects (301.5±18.42 ms) was marginally higher than that of the female (287.46±19.62 ms) subjects. The mean ART score at baseline was 247.88±14.33 ms and there was no significant difference between male and female subjects though mean score of the female subjects (250.54±26.67 ms) was marginally higher than that of the male (246.2±16.85 ms) subjects.

Test-Retest analysis of the non-intervention period values showed that the reliability in terms of reproducibility of the observations was excellent for both VRT (r =0.87) and ART (r=0.95). On the other hand, Mukha bhastrika produced an immediate and significant decrease in VRT and ART. There was a statistically significant (P<0.0001) decrease in VRT from 296.15±13.49 ms to 263.59±12.53 ms. There was also a statistically significant (p < 0.0001) decrease in ART from 247.88±14.33 ms to 217.35±11.36 ms following mukha bhastrika. There was an overall reduction of 33 ms and 30.5 ms (10.99% and 12.31% reduction) in mean scores of VRT and ART respectively after mukha bhastrika.

TABLE I : Visual reaction time (VRT) and auditory reaction time (ART) of mentally challenged adolescents before (B) and immediately after (A) performance of nine rounds of mukha bhastrika.

	B	*A*	*% Change*	*P Value*
VRT (ms)	296.15±13.49	263.59±12.53	– 10.99%	<0.0001
ART (ms)	247.88±14.33	217.35±11.36	– 12.31%	<0.0001

Values are mean±SEM for 34 subjects.

DISCUSSION

In our subjects, ART values were significantly shorter than VRT and this is in agreement with previous reports (4, 5, 10). All pre and post values obtained in the present study showed slower RT than expected values for a normal population. A literature review by Kosinski gives normal values of ART as 140-160 ms and VRT as 180-200 ms (11). In the present study the mean value of ART and VRT before mukha bhastrika was 247.88±14.33 ms and 296.15±13.49 ms respectively. This difference between the expected normal values and the values in our study can be explained by the well documented delay in the processing speed in children with MR (9).

Performance of nine rounds of mukha bhastrika produced an immediate and statistically significant decrease in both VRT and ART. The faster reactivity seen post mukha bhastrika, both in the present study as well as in our earlier study (8) may be due to a generalized alteration in information processing at the primary thalamo-cortical level that occurs during pranayama as postulated by Telles et al (6). According to the traditional wisdom of yoga, pranayama is the key to bringing about psychosomatic integration and harmony. A calm mind will be able to process information much better than an agitated one. A previous study from our laboratory has also reported a reduction in RT following three weeks of training in slow and fast pranayamas (10).

Decrease in RT signifies an improvement in central neuronal processing ability of the special children. This may be due to (i) greater arousal and faster rate of information processing (ii) improved concentration and/or (iii) ability to ignore or inhibit extraneous stimuli.

Studies done in the erstwhile Czechoslovakia have demonstrated EEC changes around somato-sensory and parietal areas of the cerebral cortex suggesting an affective arousal following agnisara, nauli and bhastrika (12). It was suggested that these practices bring about such changes through strong stimulation of somatic and splanchnic receptors. As mukha bhastrika utilizes similar forceful abdominal contractions, it may be shortening RT through similar mechanisms.

It has been reported that moderate muscular tension shortened pre-contraction RT (13) and that isometric contraction allows the brain to work faster (14). It is possible that the vigorous abdomino-thoracic muscular contractions in mukha bhastrika influenced the RT in a manner similar to isometric muscular exercise. However the post mukha bhastrika shortening of RT shows that this effect differs, as unlike muscular exercise it is carried over into the post mukha bhastrika period too.

The level of intelligence has been correlated with RT and it has been found that serious MR produces slower and more variable RT (15). In our study we have focused primarily on the mild and moderately retarded subjects with a mean IQ of 54.88±2.51. Uma et al (3) reported significant improvement of BK scores in the group having a moderate degree of MR signifying an improved IQ after yoga training. They also suggested that yoga techniques help MR children in improving their locomotor skills as well as their psycho-motor coordination.

Improved concentration and attention-span may result in improved IQ and memory power too.

RT has been found to be faster when the stimulus occurred during expiration as compared to inspiration (16). As mukha bhastrika involves multiple forceful expirations done rapidly and consecutively, this may be having a prolonged and residual neuro-muscular effect that is also influencing the RT. Mukha bhastrika may be altering afferent inputs from abdominal and thoracic regions, in turn modulating activity at ascending reticular activating system and thalamo-cortical levels. This is quite plausible as kapalbhathi, a yogic technique with similar bellows type breathing has been reported to increase mental activity and induce a calm and alert state (17).

In conclusions on the basis of the present study, we suggest that yogic breathing techniques like *mukha bhastrika* may be used as an effective means of training to improve neuromuscular abilities in special children. Further studies are required to understand the underlying mechanisms involved in bringing about such an immediate benefit.

ACKNOWLEDGEMENTS

The authors thank Dr. Madanmohan, Professor and Head, Department of Physiology and Programme Director, ACYTER, JIPMER for his support at all stages of the study. We thank Yogacharini Meenakshi Devi Bhavanani, Director ICYER for her constant motivation, encouragement and supportive guidance for this work as well as yoga training for special children. We thank Dr. M Sugatham, Consultant psychologist and Director SADAY School for Special Needs permitting us to do this study and for encouraging the yoga therapy sessions for the children for the past 5 years. We also thank the staff members of SADAY for their cooperation and assistance and Dr. R Balaji, Junior Faculty member of Yoganjali Natyalayam for his valuable assistance in the recordings.

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LETTER TO EDITOR

BLOOD PRESSURE MULTI-FACTORIAL INFLUENCE AND TRENDS IN INDIAN MEDICAL STUDENTS

(Received on May 5, 2010)

Sir,

Increasing prevalence of hypertension in the adolescents worldwide is now a major concern. Hence, we attempted to study the prevalence of hypertension in adolescent Indian males.

A study was conducted on 228 first year undergraduate male medical students during the year 2007-09 in a medical college in Delhi. Each student filled up a questionnaire recording his age, diet and family history of hypertension. Weight and height was measured to calculate Body Mass Index (BMI). Using the BMI criteria of WHO (1), the body types were categorized as underweight (BMI <18.5), normal (18.5-24.9), overweight (25.0-29.9) and obese (>30). Blood pressure was measured and classified as per the Seventh Report of the Joint National Committee where Pre-hypertension is systolic BP (SBP) of 120-139 mmHg or diastolic BP (DBP) of 80-89 mmHg and Hypertension stage 1 is SBP of 140-159 or DBP of 90-99 mmHg (2). Subjects were informed about the study and their voluntary written consent was taken.

Mean BP were computed for weight, height, BMI and blood pressure, data was analysed using chi-square test to find association between hypertension and variables (BMI of <25 & BMI >25, diet – vegetarian and non-vegetarian and presence or absence of family history of hypertension). Those found to be significantly associated with hypertension ($P<0.05$) were then entered in multiple logistic regression (Enter method was used).

According to JNC-VII criteria, 21% of the students were pre-hypertensive while 2.6% were stage 1 Hypertensive. Table I depicts the values of different variables with respect to the hypertension stage. Since the Stage 1 hypertension had only 6 subjects, we clubbed the subjects of pre-hypertension and stage 1 hypertension into a single group for appropriate statistical analysis. Table II shows the respective P-values and Odds Ratio

TABLE I : Values of different variables with respect to different stages of Hypertension.

n=228	*Normal BP*	*Pre-HT*	*HT-Stage 1*
n (% of HT)	174 (76.3%)	48 (21%)	6 (2.6%)
BP (Mean±SD)			
SBP mmHg	112.21±4.67	126.68±5.72	143.6±4.76
DBF mmHg	73.86±4.75	82.62±2.58	93.76±2.77
BMI (n) kg/m^2			
<25	131(57.5%)	26(11.4%)	2(0.8%)
≥2S	42(18.4%)	23(10%)	4(1.8%)
DIET (n)			
Veg	118	18	2
Non-veg	54	31	4
Family Hist. (n)			
Present	53	23	3
Absent	120	26	3

HT = Hypertension

TABLE II: Respective P-values & Unadjusted Odds Ratio for different variables.

Variable	*Code*	*n & % of HT*	*Unadjusted OR (95% CI)*	*P-value*
BMI kg/m^2	<25 = 0	(n=159) 28(17.6%)	1	
	>25 = 1	(n=69) 27(39.1%)	3.008(1.598–5.662)	<0.001
DIET	Veg = 0	(n=138) 20(14.5%)	1	
	Non-veg = 1	(n=89) 35(39.3%)	3.824(2.023–7.229)	<0.001
Family history	Absent = 0	(n=149) 29(19.5%)	1	
	Present = 1	(n=79) 26(32.9%)	2.030(1.092–3.774)	0.025

HT = Hypertension, OR = Odds Ratio, CI = Confidence Interval.

for BMI, diet and family history. All three variables were found to be significant after the application of Chi-square test. After applying multiple logistic regression, only BMI & diet was found to be significantly associated with hypertension.

High prevalence of hypertension in the adolescents could be because majority of the study population belongs to upper middle or middle socio-economic status, where there is an altered eating habits and increased fat contents in the diet. Most of them give up their sports and active lifestyle long before, during their school life, in their quest to pursue a medical career and lead a sedentary lifestyle with an addition of mental stress to get through the competitive medical entrance exam, some of the contributory factors which cannot be overlooked upon (3). Our study showed BMI >25 has 3 times more likelihood of developing hypertension as compared to BMI <25. Mohan and Uchiyama also observed an increasing prevalence of sustained hypertension in the obese younger age groups as compared to their lean counterparts (4, 5).

This study also emphasizes upon the association of diet with BP. Non-vegetarians showed 3.7 times more likelihood of developing hypertension. Consumption of food of animal origin is highly significantly associated with increase in BP (6). Study by Melby reported that only 16% of the vegetarians were hypertensive compared with 31.1 % of the non-vegetarians (7). Vegetarian diets have a relatively high polyunsaturated to saturated fat ratio, are low in total fat and has high fiber content- having the tendency to reduce body weight and modulates blood viscosity, along with the BP-lowering properties of individual nutrients (8).

Family history of hypertension also plays a pivotal role in the development of hypertension. Our study showed significance of family history at 8% level and those with positive family history were 1.8 times more likely to be hypertensive, similar to the study by Young (9). This association could be due to an impairment in baroreflex sensitivity in hypertension which in part is genetically determined (10). Higher levels of angiotensinogen, cortisol and 18-OH corticosterone seen in the offsprings of high parental blood pressure may also lead to abnormalities of glucocorticoid metabolism and the renin-angiotensin system (11).

In conclusion, our study emphasizes upon the alarming prevalence of pre-hypertension

in the adolescent Indian males which can be attributed to various factors like weight, BMI, dietary habits and family history. Early modification in these variables can be very useful in decreasing the future prevalence of hypertension.

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LETTER TO EDITOR

ELEVATED BLOOD LEAD LEVEL DESPITE DISCONTINUATION OF LEADED PETROL

(Received on June 17, 2011)

Dear Editor,

Worldwide, lead (Pb) ranks as one of the most serious environmental poisons. Chronic lead exposure often does not result in overt manifestation of toxic symptoms but leads to slow, progressive and usually irreversible toxicity of hematopoietic, nervous, renal, gastrointestinal and reproductive system. Lead pollution due to its adverse effects on health remains a public health concern in developing countries such as India, where use of leaded petrol has recently been phased out. However, lead continues to be found in other sources like pipes carrying drinking water, paints, canned foods, glazed ceramics, herbal medicines, batteries, cosmetics, jewellery etc. Lack of awareness about the sources of lead and ill effects results in health hazards to many adults and children causing significant economic damage.

In this context, we refer to the letter to the Editor titled "Effect of environmental lead pollution on hemoglobin and erythrocyte ALAD activity" by Sharma et. al. published in Indian J Physiol Pharmacol 44(1): 117-118, 2000. The authors had conducted a study to examine the effect of lead exposure on blood indices of traffic policemen and reported a significantly higher Blood Lead Level (BLL) and decreased erythrocyte ALAD (Delta- aminolevulinic acid dehydratase) activity in traffic police personnel who were supposed to be exposed to higher vehicular pollution as compared to the control group. It is now over a decade that leaded petrol has been discontinued in India; therefore, we have once again examined the impact of discontinuation of lead containing petrol on BLL and related biochemical parameters in the normal population of Jaipur.

The present study comprises of 250 subjects, of which 141 were from rural and 109 were from urban background, 217 were male and 33 were female, ranging in age from 20 to 70 years. Fasting blood was collected from the antecubital vein in EDTA vial by taking all aseptic precautions. Blood was analyzed for erythrocyte ALAD activity (2) immediately and a small aliquot of blood was diluted with nitric acid - Triton X-100 solution and analyzed for BLL using atomic absorption spectrophotometer (3) by the same methodology as adopted by Sharma et. al. The results of our study are shown in the table below.

The mean BLL of 250 subjects in the present study was found to be 151.6±118.2 ng/ml. The urban population had a significantly higher ($P<0.001$) BLL (232.3±98.0 ng/ml) as compared to the rural population (89.3±71.8 ng/ml). A significantly decreased ($P<0.001$) erythrocyte ALAD activity (34.47±17.28) was observed in the urban population as compared to the rural

Table I: Biochemical characteristics of normal healthy subjects.

Parameters		*Total (n=250)*	*Rural (n=141)*	*Urban (n=109)*
Lead (ng/ml)	Mean±SD	151.6±118.2	89.3±71. 8	232.3±98.0
	Range	1.0–397.1	1.0–372.0	2.0–397.1
5-ALAD (U/L)	Mean±SD	37.89±16.33	40.53±15.10	34.47±17.28
	Range	6.63–78.23	8.60–77.56	6.63–78.23

population (40.53±15.10). The increased BLL in the urban population could be attributed to the continuous use or exposure to various sources of lead in day to day life. It is observed that 58.8% of the urban population was found to have BLL above 10 ug/dl (100 ng/ml) which is prescribed as the safe cutoff by WHO (4).

In the present study, the mean BLL of the urban population continues to be significantly higher (232.3±98.0 ng/ml) than the safe limit prescribed by WHO and is comparable with the findings of Sharma et al (275.9±53.4 ng/ml) reported more than a decade ago (1). This finding is quite surprising considering that it is now over a decade that leaded petrol has been discontinued in India. This indicates that, discontinuation of lead containing petrol alone could not improve the situation. There appears to be many environmental, occupational and nutritional factors responsible for lead pollution in the environment and elevated blood lead level. This is a matter of serious concern.

In view of this, it has been recommended that, we need to launch a mass education and awareness programme about the ill effects of chronic lead exposure, screen the population for BLL, monitor environmental lead level and develop methods to eliminate lead from various sources.

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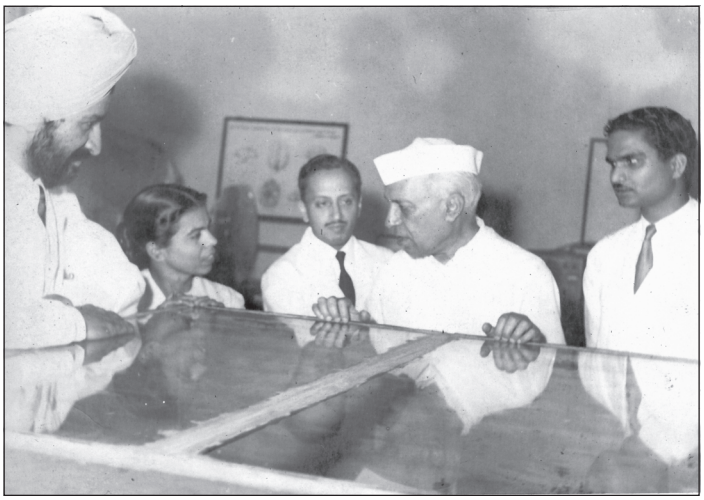
Obituary

Dr. Gulzar Singh Chhina
(1926–2011)



Dr. G.S. Chhina (extreme left) with former Prime Minister late Pandit Jawahar Lal Nehru, when he visited the Department of Physiology, AIIMS. Others in the photo are Dr. (Mrs.) S. Dua Sharma Dr. A.S. Paintal, and Dr. A.P. Sharma.

Dr. Gulzar Singh Chhina passsed away on 03 Sep 2011, at his residence at Swasthya Vihar in Delhi, following a short illness. With his demise an era in the history of Indian physiological science came to an end.

Dr. Chhina came from a humble rural background. His father, Arjan Singh, had served in the British Indian Army and was decorated for gallantry in Mesopotamia (Iraq) during the First World War. After the war he was given a piece of land as a reward for war services, in the newly established canal colony of Lyallpur (now Faisalabad in Pakistan). The family then moved to Lyallpur from their ancestral village of Harsha Chhina in Amritsar District. The surname of Chhina comes from the name of his ancestral village. Gulzar Singh Chhina was born in Lyallpur on 10th April 1926. He was the youngest of four siblings, viz. a sister and two elder brothers. As a child, he was educated in a village school, and he had to cycle five miles to the primary school situated in the next village. He lost his father at the age of 12, and was very close to his mother, Balwant Kaur, who, along with his elder brothers and sister, laboured hard to ensure that his education did not suffer despite the enormous family setback and the loss of everything that they owned, after the partition.

Dr. Chhina graduated in Physiology and Anatomy in 1951 from Amritsar Medical College, and did his post-graduation in Physiology in 1954, from the same college. He started his career as a Lecturer in Physiology at Punjab Government College of Physical Education. The turning point in his life came in 1955 when he decided to quit his government job to join as a Research Fellow of Indian Council for Medical Research (ICMR) under Dr. B. K. Anand in the Department of Physiology at Lady Hardinge Medical College, New Delhi. In 1956, the All India Institute of Medical Sciences (AIIMS) was established at New Delhi, and Prof. Anand was appointed as the Head of the Department of Physiology. In 1956, ICMR granted a Neurophysiology Research Unit to Prof. Anand, with Dr. Baldev Singh as

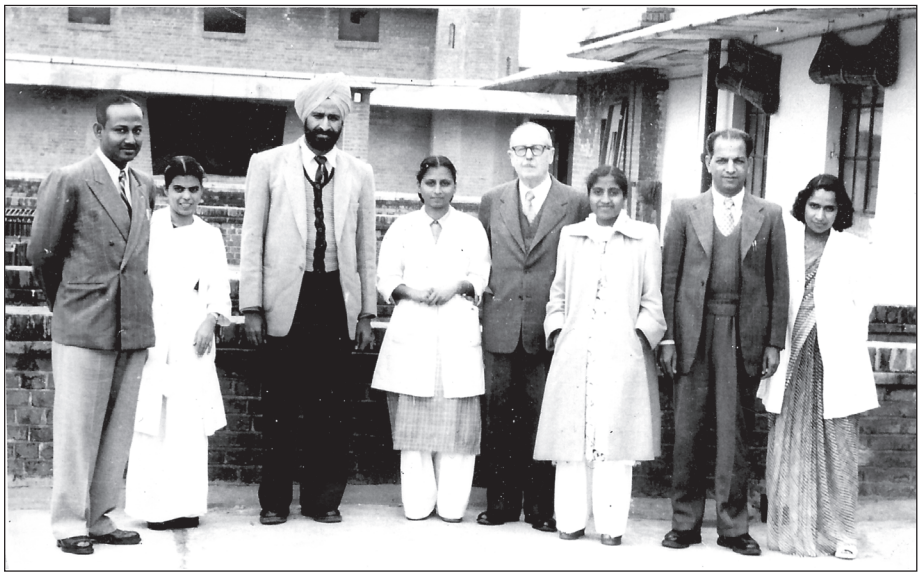
collaborator. This unit was transferred to the AIIMS, and all those who were working with Dr. Anand also moved over to AIIMS to join this research unit. Dr. Chhina's association with Dr. Anand and Dr. Baldev Singh, the two doyens who pioneered the study of neurosciences in India, continued till the end of their lives. In 1957, he also had the good fortune to work with Dr. M.A. Wenger, Professor of Psychology, University of California, Los Angeles, USA, and Dr. B.K. Bagchi, Professor Electrophysiology, Michigan University, Ann Arbor, ILL, USA, and learn electroencephalography, electrophysiology and psychophysiology from these most renowned experts in the field at that time. Working under the guide-ship of Dr. Anand, he did his PhD in Physiology in 1960 from Punjab University and AIIMS. His thesis work titled "Role of the limbic system of brain in the regulation of affective behavior" should be considered as a bible for anyone stepping into the field of neurophysiology.

Dr. Chhina married Kanwar on 22 April 1956. His strong views on the equality of the sexes caused a flutter during his marriage ceremony. He insisted that his bride should lead during the phera ceremony. This procedure was stalled until a compromise was worked out by which both the bride and groom walked around the Holy Granth side by side. In 1960 Dr. Chhina was blessed with a son.

In 1961, Dr. Chhina was awarded a post-doctoral fellowship of the National Institute of Health. He worked for a year from Jan 1962 with Dr. John Brookhart, Chairman and Professor of department of Physiology, Medical School, University of Health Sciences, Portland, Oregon, USA. There he learned several techniques in Neurophysiology, and carried out experiments on conscious unrestrained freely moving cats to record single neuron activity of the visual cortex, lateral geniculate and visual pathways. He also used stereotaxically guided metal and glass microelectrodes with remote controlled microdrive.

Dr. B.K. Anand was very fortunate in getting some excellent devoted youngsters like Dr. Chhina to work with him. Dr. Anand discovered the "feeding centre" in the hypothalamus while working with Prof. John R. Brobeck at Yale University in USA. But many details of the control mechanism of food intake were worked out by his junior colleagues like Dr. Chhina in India at AIIMS. Dr. Chhina not only worked on hypothalamic regulation of food intake and limbic system regulation of reproduction, but did some pioneering studies on yoga, which continue to be quoted even today. Studies done in collaboration with Dr. Anand and Dr.Baldev Singh, on Shri Ramanand Yogi during the yogi's stay in an air-tight box, and published in 1961, are a pioneering scientific work in the field of Yoga. Through his persistent and devoted work he climbed up the ladder to become a full professor of Physiology at AIIMS in 1976. Later on he became the head of the department.

Of all those who shaped his career, one has to mention the name of Dr. Baldev Singh. Dr. Baldev Singh who was collaborating with the Neurophysiology Research Unit, in all its activities, joined as a Professor and Head of Department of Neurology at AIIMS, in Feb 1965. After retiring from the Department of Neurology in 1968, he joined the department



Dr. G.S. Chhina with other colleagues (From left Dr. Sen, Dr. S. Dua Sharma, Dr. G.S. Chhina, Dr. Santosh Mahindra, Dr. B. Hussey (visitor), Dr. B. K. Maini, Dr. B.K. Anand and Dr. Subhadra Kuldeep; Venue : Lady Harding Medical College, New Delhi).

of Physiology, AIIMS, as Emeritus Scientist of ICMR and Emeritus Professor of AIIMS. Dr. Chhina had his room in the department of Physiology, which was adjoining that of Dr. Baldev Singh. There was an interconnecting door between the two rooms, and Dr. Baldev Singh was most often found in Dr. Chhina's room. Dr. J.S. Bajaj, who was the Professor of Medicine and Dr. P.N. Tandon, who was the head of the department of Neurosurgery at AIIMS, would often join them during late evenings. Their interactions had interactions had resulted in many collaborative research projects of national interest. Dr. Chhina used to have a stream of visitors who would come to him to seek his advice on various scientific matters. Dr. Baldev Singh also used to have his quota of visitors who would come to him to get his opinion on scientific issues. During their discussions, if Dr. Baldev Singh felt that it was necessary to get a second opinion, he would bring the guest to Dr. Chhina's room for further discussions.

It was very rare for Dr. Chhina to leave the department before dinner time. If there were visitors in his room for Dr. Baldev Singh, he would disappear into his laboratory, and continue his work there. His laboratory (which was called electronics lab - for some strange reason) had a small cabin with a couch. This was used for human sleep studies. He would relax on the couch and get on with his reading, unmindful of the hustle and bustle going on outside the lab. Many a times, students would be working and talking in the laboratory without realizing that Dr. Chhina was inside the cabin.

Though Dr. Chhina had guided many students, he was made a full guide for PhD only

in 1968. Fortunately I happened to be his first student in that capacity. Dr. Anand was my co-guide. Dr. Chhina was very fond of his laboratory and instruments, to the extent of being very possessive about them. He was very good at instrumentation and surgery. He never used to allow the new students to touch the instruments in his absence. As one could imagine, it was a frustrating experience for all those who used to work under him. At that time we could never appreciate his attachment towards the laboratory, and the effort that he had put in to bring it to that level of excellence, which was at par with any leading laboratory in the world. The lab had oscilloscopes, amplifiers, pulse generators, wave form generators, power supplies and several electronic equipments from Tektronix and Grass companies. The instruction manuals of all the equipments were under lock and the key, and were in the safe custody of Dr. Chhina. We students who were seeing those equipments for the first time in our lives, found it fascinating to watch him manipulate the instruments, and were greatly tempted to do the same thing ourselves. One day some of us, including a technician, decided to lock the lab from inside and manipulate the instruments ourselves. I cannot describe the thrill that we had when we manipulated the various knobs of the instruments. The technician also joined us in the rejoicing and said that "The Sardarjee would have a shock of life if he comes to know that we have switched on these equipments. He would not even show us the manual."

Suddenly the door of the small cabin inside the lab opened and the huge towering figure of Dr. Chhina appeared before us. One can imagine our plight at that time. Dr. Chhina did not utter a word. He went towards the equipments, pulled out a stool, sat in front of the oscilloscope and started explaining the functions of each and every controlling knob. Most of his explanations, at that point in time, went above our heads. But we did not have the courage to ask for any explanation. At the end he asked us whether we have understood every thing. We all kept silent. What he said after that is still vivid in my memory. "All these equipments are for you only. Who will be the sufferer if something goes wrong? I will be sad or even mad if something goes wrong, but you who have to use these equipments for your thesis work will be the real sufferer. There is nothing more precious than the instruction manual. Probably it is more precious than me and my equipments. Even if I don't teach you, you can learn everything from the manual. No one taught me the use of these equipments. I learned it all from the manual. In fact, I do not want to teach you. I want you to learn it all yourself. Before I give the manual, and before I let you touch the equipment, I want to have confidence in you. I will give you the manuals one by one. You cannot take them home or to your hostel. You will have to sit with the manual in front of the equipment, and go step by step. You will have to return the manual at the end of the day". I should mention here that photocopying was not available on those days, not to speak of internet and website, from where one can download the information.

Dr. Chhina was a highly principled person who followed the Sikh religion. He had great respect and regard for all religions. He was a teetotaler and was not fond of non-vegetarian food, unlike many from his community. Talking about his community, I am reminded of a foolish mistake I had made during my initial days of stay in Delhi. For those who follow

Sikh religion, it is considered a great sin to smoke. Born and brought up in south India, I was totally unaware of this aspect of Sikh religion. There was a departmental party, a few days after I had joined AIIMS. The host was passing around a box containing imported cigars. Even those non-smokers were examining the cigars and were singing praises of that rare quality stuff. In an effort to please my boss, I took the cigar box and walked briskly towards Dr. Chhina to offer them to him. Stunned by the action, the rest of the crowd ran towards me, and snatched the cigar box from my hand. This foolish act of mine was a subject for laughter during the rest of the party. Later people explained to me about the customs and practices of Sikh religion. If I had been in Dr. Chhina's shoes, I would have been telling this joke at every opportunity. But, I have never heard him telling this joke to anyone.

Dr. Chhina had a well-rounded personality. He could read and write Persian, Urdu and Punjabi. He was also very fond of Urdu poetry, and he used to sing a few couplets during get-togethers and parties. He was a very humble, simple and dedicated person with unflinching devotion to his first love, i.e. neurosciences and neuroscientists. He had exemplary ability to understand and analyze scientific problems. His knowledge and love was never limited to physiology and medical sciences. He had good knowledge of subjects like electronics, physics and psychology. In all scientific meetings, people used to look forward to his suggestions and comments. He always encouraged and supported hard working sincere students, even if they were not working under him. He never tolerated any student who took a short cut towards success. He never went out of the way to support his own students, if they were not deserving. This was something which distinguished him from the rest of the faculty. As students we could never appreciate this quality of his. But now, looking back I can admire the strength of his character. He never strove for cheap popularity among students, unlike many of his colleagues.

After retirement from the department of Physiology in 1986, he joined as Emeritus Medical Scientist in the department of Neurology of AIIMS. After a year he went to Iraq to work as Visiting Professor of Physiology in the Military College in Baghdad, and later as Professor of Physiology at Basra. In 1990 he went to USA and joined as Director Research at Tele-health Corporation, Baltimore. While in USA, his expertise in yoga and knowledge of alternative medicine was sought after by various scientific bodies like SKY Foundation of Philadelphia, Himalayan International Institute of Yoga Science and Philosophy, Honesdale, and National Institute of Health. He used to attend the Neuroscience meetings, and was a member of the Society of Neuroscience, USA. His former students and admirers used to make it a point to meet him during the Neuroscience Congresses. During his stay in USA, he used to visit India frequently. His wife's failing health and that of his own, made him spend more time in India, after 2000. He finally returned to India in Oct 2006, but he kept himself active by staying in touch with professional and scientific activities. I remember very well the keenness with which he attended the National Sleep Medicine Course, which was conducted at India Habitat Centre, Delhi, on December 12-13, 2009. He participated in all the deliberations and discussions, as he used to do several years back.

The year 2010 was a tragic one for Dr. Chhina. Mrs Kanwar Chhina passed away on 27 August 2010. She was like the rock of Gibraltar in his life, always giving him her full and unstinting support for over 54 years. Her demise was a huge blow for him as no one could replace the companionship that she had provided him throughout their lives together. He survived the passing of his wife and lifelong companion by one year and one week. In early August 2011, Dr. Chhina went to AIIMS for a routine check-up, but suffered a stroke whilst there. He never fully recovered from it. He spent his last few days at his residence at Swasthya Vihar in Delhi, and passed away peacefully on the afternoon of the 3rd September 2011.

We can best honour his memory by emulating his unbending spirit and courage of conviction in academic pursuits. He will continue to be an unlimited source of inspiration for all. His timeless personality will keep on inspiring the future generations to pursue the unending task of shrinking the unknown and expanding the span of knowledge. May his soul rest in peace in heaven with the satisfaction that the seed of interest in neurosciences that he had sown is being nurtured in the minds of his admirers.

Finally, I will be failing in my duty if I do not thank Rana who provided me with a lot of material that appears in this obituary.

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Report of 57th Annual Conference of Association of Physiologists and Pharmacologists (APPICON 2011)

The 57th Annual Conference of Association of Physiologists and Pharmacologists of India was held from 13-17th December 2011 at the All India Institute of Medical Sciences, New Delhi. The conference was attended by more than 900 delegates from all over the country and abroad. The conference was graced by about 100 Invited Guest Speakers of National and International repute. The conference truly represented the length and width of the country with delegates from all states barring one of India with representation from Maharashtra (142) and Karnataka (116) dominating in numbers. The vibrancy of the APPI was also revealed in record 550 plus abstract submissions from all the important sub-fields of physiology, namely, Neurosciences (16%), Metabolism (13%), Endocrinology (12%), Cardiovascular (11%), Complementary and Alternative Medicine (11%), Respiratory Physiology (10%), Medical Education (4%), Hematology (3%), Phytomedicine (3%), Environment (2%). The pharmacology section contributed to 6% of abstracts. The pride of APPI reflected in very high representation by postgraduates and Junior Faculty.

The conference was held for 2 and ½ day as per the constitution of APPI with one day of Pre-conference "Young Scientist Training Workshop of Techniques and Tools" (six workshops) supported by Department of Science and Technology and one day of International Symposium on Systems Biology. The systems biology symposium covered an important emerging field and was addressed by International faculty from all over the globe. The workshops covered techniques on cell culture and molecular biology, cardiovascular physiology and pharmacology, autonomic physiology, electrocencephalography and integral health management and practice of yoga. The Guest faculty from various part of the country shared their views during the workshops.

The main conference included Plenary Session and Inauguration, Orations, 10 symposia, 10 thematic sessions for free paper presentation (110 papers), 2 sessions of thematically categorized poster presentation (440 presentations), 3 parallel Award Sessions for undergraduate, postgraduates and APPI awards and a special session to discussion the VISION of APPI for next 10 years. The poster presentations were done in group of 10-15 sub-sessions (40 sessions) that chaired by scientists of repute. Forty full length submissions were made for awards for best research presentation by postgraduates and undergraduates out of which 16 were selected for presentation during the conference. The symposia topics covered feeding behaviour, reproductive physiology, high altitude, neurodegeneration and plasticity, biologics, cardiovascular pharmacology, lifestyle and yoga, space travel, sleep and cognition. The symposia were addressed by Guest Faculty from all over the India and other countries. The Session of VISION 2020 reiterated the ethical issues in human and animal experimentation, and need for publication, mentorship and popularizing physiology and pharmacology amongst medical students and general public.

The executive committee meeting was held during the conference. The editorial board for the Indian Journal of Physiology and Pharmacology were formally elected (2012 - 2015). The General body meeting was held on December 16, 2011. It was unanimously agreed to hold the APPICON 2012 at Netaji Subhash Chandra Bose Medical College, Swami Vivekananda Subharti University, Meerut.

First Announcement – APPICON 2012
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