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EDITORIAL

e- waste and our responsibility.

Prof. Dr. Parimal Chandra Sarker

Electronic waste or e-waste are the discarded electrical or electronic devices after the end of their useful life. These are one of the fastest growing hazardous waste in the world and estimated that about 50 million tons of E-waste are produced each year[1]. The rapid changes in technology, changes in media like tapes, software, MP3, more over falling of prices, and planned to change obsolescence have resulted in a fast-growing surplus of electronic waste around the globe[2]. The European Community of Waste Electrical and Electronic Equipment Directive (WEEE Directive) classifies e-waste in ten categories: (i). Large household appliances (including cooling and freezing appliances),(ii). Small household appliances,(iii). IT equipment (including monitors),(iv) Consumer electronics including TVs,(v). Lamps and Luminaires, (vi)Toys, vii.Tools, (viii) Medical devices, (ix).Monitoring and control instruments and (x) Automatic dispensers[3].

Whatever the sources of the e-wastes they follows a range of pathways after their disposal, including formal and informal recycling, storage and dumping in both developed and less-developed country contexts[4]. The waste comprises a variety of hazardous materials some are potentially valuable and recyclable and other inputs a great health hazards[3,4]. The processes of dismantling and disposing of electronic waste in developing countries led to a number of environmental impacts also. Different liquids and atmospheric releases of the electronic end products are polluted ground water, soil, air and therefore in the cultivating land , river and sea. Dioxin an airborne pollutant which is found at 100 times greater levels as previously measured[5].

Levels of carcinogens in pond water, river water and rice, paddies and other food grains exceeded international standards for agricultural areas. More over cadmium, copper, nickel, and lead levels in rice and paddies became above the international standards. Heavy metals like lead found in road dust over 300 times that of normal[5].

The electronic waste are regularly introduced with consumption of crops grown in the soil which is eaten by human, and through in drinking water. Not only human all the animals –both domesticated and wild, and the poultry birds also affected in the same ways[6]. Polychlorinated biphenyls (PCBs) from e dust can be absorbed via skin or inhaled or by ingestion causing neurotoxicity, liver damage, different tumors, immunosuppression, behavioral changes, and reproductive disorders[7]. Another component of e-waste like Polybrominated diphenyl ethers (PBDEs) is an environmentally persistent compound, which is also reported as a bioaccumulation product. It causes abnormal brain development of a fetus, associated with impacts on learning, loss of memory and behavioral changes[8]. When PBDEs are burnt they produce brominated dioxins/furans which are hazardous and Triphenyl phosphates (TPP) are a contaminant in human produces many inhibitory action on human blood cells[9].

Heavy metals such as Lead, Cadmium,

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Antimony, Murcury, Barium, Beryllium is may produce irreversible effects in nervous system, respiratory system, cardiovascular system, reproductive system and kidneys in children[10]. Antimony is a toxic compound causing dermatitis, affecting skin cells and respiratory tract and affects the immune mechanism. Cadmium is a toxicant which can accumulates in tissues, exposure may affect kidneys and bones and it disrupts calcium mechanism, causing hypertension and heart diseases Cadmium oxide in fume affects the respiratory system also[11].

So, the disposal and recycling of electronic waste routinely entails the movement of hazardous materials in the environment in the national and across national borders. Globally, the handling and regulation of e-waste as both a hazardous waste stream and as a source of secondary raw materials[12]. A growing number of countries have adopted extended producer responsibility laws, which mandate electronics manufacturers to pay for proper recycling and disposal of electronics. The Environmental Protection Agency estimates that only 15–20% of e-waste is recycled, the rest of these electronics go directly into landfills and incinerators[13]. While there are many additional steps that can be taken in order to ensure that recipients of waste are adequately prepared to manage and recycle them in an environmentally sound manner[14]. As these developments unfold, regulation and oversight will play a decisive role in mitigating the myriad risks to human health and the environment that can result from e-waste recycling[15].

It is very necessary to produce a definite rule for manufacturer, distributer and consumers for the use and disposal of electronic appliances. The consumers should informed about usefulness of their devices as well as their disposal when obsolete these electronic devices. The consumers should be advised to deposit their e-waste to stores or collection centers. Similarly, manufacturers and assemblers of the devices will have to collect e-waste either for reuse or destruction during production of electrical and electronic goods and ensure delivery of the e-wastes to the registered repairers, dismantlers and recyclers, according to the draft rules on management of wastes generated from electrical and electronic goods. As the e-wastes often rich in precious metals and other reusable materials such as plastics, are also perceived as a source of additional income. More over proper protection of the workers needed who will be involved in e-waste handling, dismantling and recycling as there are at risk of carcinogenic and non-carcinogenic health hazards.

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Original article

Evaluation of Parasympathetic Nerve Function by Heart Rate Response to Valsalva Maneuver in Overweight Individuals

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ABSTRACT

BACKGROUND: *Overweight individuals are increasing at an alarming rate throughout the world and this has been called "New world syndrome". Overweight is an important risk factor for non-communicable diseases and associated with parasympathetic nervous system dysfunction.* ***OBJECTIVES:*** *To assess parasympathetic nerve functions by heart rate response to valsalva maneuver in overweight individuals.* ***METHODS:*** *This cross sectional analytical study was conducted from July 2017 to June 2018 in the Department of Physiology, Rangpur Medical College, Rangpur. After obtaining permission the subjects who would meet the inclusions and exclusions criteria were enrolled in the study after briefing them about objectives. A total number of 80 age, sex matched individuals were selected, among them 40 were apparently healthy normal weight individuals (group A) and 40 were apparently healthy overweight individuals (group B). The evaluation of parasympathetic nerve functions in overweight individuals were done by performing valsalva maneuver test. The subjects were selected from different areas of Rangpur division. For statistical analysis, unpaired t test was performed by computer based software SPSS 23.0 version for windows.* ***RESULTS:*** *Heart rate response to valsalva maneuver was significantly lower in overweight individuals ($p < 0.05$) than those of control.* ***CONCLUSION:*** *The study thus concluded that decrease heart rate response to valsalva maneuver test in Overweight individuals indicate in the parasympathetic nerve dysfunction.*

KEY WORDS: Overweight, valsalva ratio, parasympathetic nerve function.

INTRODUCTION

Increased weight gain has become a major health concern in the modern society, probably as a byproduct of rapid industrialization and sedentary lifestyle[1]. In Bangladesh, 7% adults were overweight or obese in 1980 but those rates had claimed to 17% in 2013[2]. Overweight and obesity are defined as abnormal and excessive fat accumulation that may impair heath[3]. Overweight and obesity are the fifth leading preventable causes of death worldwide[4]. Globally 4.5 million deaths were estimated due to complication caused by overweight and obesity[5]. The measurement of body fat is too complex to be of practical clinical application. Body mass index measured in Kg/m^2 provides the most useful population level measure of overweight and obesity as it is same for both sexes and for all ages adults[6]. The world health organization recommends the cut off values for the

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definitions of overweight and obesity should be lower for Asian populations as follows[7]:
Normal weight : BMI 18.5 to 22.9 kg/m^2,
Overweight : BMI 23 to 24.9 kg/m^2,
Obese : BMI $\geq$ 25 kg/m^2.

Overweight and obesity negatively affects body strength, lower body mobility and daily activities. Aside from physical limitation overweight and obesity are major determinant of chronic illness[5]. Autonomic nerve dysfunction also co-exist in addition to recognized complication caused by overweight and obesity which is related to an increased risk of hypertension, cardiovascular diseases, diabetes mellitus and sudden death8,[9]. The autonomic nervous system (ANS) is responsible for regulation and integration of internal organs functions through its two branches: the sympathetic and parasympathetic nervous system10. Cardiac parasympathetic dysfunction present in overweight and obese individuals could be associated with higher carbohydrate intake[11].

The assessment of ANS requires recording the response of an end organ to physiological maneuvers. The heart rate responses are indicative of cardiac parasympathetic integrity[12]. The simple, reliable and non invasive valsalva maneuver tests based on cardiovascular reflexes is useful tool for evaluating parasympathetic modulation of the heart. Normally heart rate varies continually but this depends on an intact parasympathetic nerve supply. Responses to the valsalva maneuver depends on efforts and the heart rate rises during the period of straining. Lower response to valsalva maneuver occurs in overweight individuals[11,13].

Overweight and obesity related health problems are not uncommon in our community[8]. Its health consequences ranges from increased risk of premature death to serious chronic condition that reduce the overall quality of life[14]. So, the autonomic functions can be investigated in asymptomatic overweight individuals to identify any derangements in the latent subclinical phase itself. This might be help in early diagnosis and prevent further complications associated with overweight[1].

MATERIALS AND METHODS:

This is cross-sectional analytical study was conducted in the Department of Physiology, Rangpur Medical College, Rangpur from July 2017 to June 2018. A total number of 80 apparently healthy subjects, aged 20-40 years were included in this study from different community of Rangpur division. Before perform the parasympathetic nerve function tests and collection of sample all the subjects were briefed about the objectives of the study. Informed written consent of the study subjects were taken in easily understandable Bengali phrases. A standard questionnaire was filled up after taking history and thorough clinical examinations.

All the study subjects were divided into two groups on the basis of their body mass index, among them 40 normal weight inividuals was denoted as group A and 40 overweight individuals was denoted as group B. They were matched with age, sex and socioeconomic condition. Study was carried out with prior protocol approved by the thesis protocol review committee and ethical committee of Rangpur Medical College. Obese and history of Diabetes mellitus, Chronic renal failure, Hypertension, Cardiac complication, Chronic obstructive lung diseases, Smoking and alcohol abuse, Any Neurological disorder, Psychiatric disorders (Depression) taking any neurotoxic

drug, Endocrine disorders (thyroid, adrenal etc) and Previous history of head injury were taken as exclusion criteria.

For calculation of BMI weight in Kg and height in m2 of each subjects was taken by medical weighing machine and measuring tape respectively.

From the previous night up to the examination, they should not undergo any physical or mental stress and not to take any sedatives or any drugs affecting central nervous system. Then the autonomic nerve function parameters were assessed by cardiac reflex tests using sphygmomanometer and ECG machine. Heart rate response to valsalva maneuver tests was conducted in a comfortable environment in the departmental physiology laboratory from 9.00am-2.30pm.

Resting pulse rate15:

The subjects were asked to take rest for 10 minutes in supine position and radial pulse rate was recorded in supine position and expressed as beats/min. Three reading were taken and the average of the three was taken as the resting pulse rate.

Heart rate response to Valsalva maneuver:

The test were performed by asking the subjects to lie down on the bed and then to blow into a rubber tube connected to a sphygmomanometer to keep the mercury up to a height 40 mm of Hg by continuous blowing and the level maintained at the mark for 15 seconds while a continuous electrocardiogram was recorded. Record for 30 seconds more after this maneuver with patient sitting and strip ECG recording. Repeat twice, and average the ratio from the 3 Valsalva attempts was taken to calculate valsalva ratio.

Valsalva ratio:

Ratio of longest R-R interval within 20 beats of ending maneuver to shortest interval during maneuver, measure with a ruler from electrocardiogram trace. Normal: $\geq$ 1.21, Borderline: 1.11-1.20, Abnormal: $\leq$1.1

All data were recorded systematically in a preformed history sheet and all statistical analysis was done by computer using the software SPSS 23.0 version for windows. Comparison between the experimental group (overweight and obese subjects) with control group (normal weight) was done by unpaired t test. In the interpretation of results, < 0.05 level of probability (p) was accepted as significance.

RESULT:

The mean ± SD of age were 29.1500 ± 6.33489 years in group A and 30.700 ± 7.62990 years in group B. The mean ± SD of height was 1.6168± 0.12711 m in group A & 1.6525 ± 0.09021 m in group B and the mean ± SD of weight was 56.1500 ± 8.53214 kg in group A, 63.0650 ± 8.08321 kg in group B. The mean ± SD of BMI were 21.39 ± 0.95149 kg/m2 in group A, 23.6275 ± 0.54112 kg/m2 in group B. The mean ± SD of WC were 71.8250 ± 6.5511 cm in group A, 86.700 ± 5.2242 cm in group B. The age and height were statistically non significant between the groups but weight, BMI are very highly significant in group B than group A (Table – I).

Table - I: Showing mean ± SD of age, height, weight and BMI of the study subjects in group A and group B.

Group	Age-years	Height-m	Weight- kg	BMI-kg/m2
Group A n= 40	29.15±6.33 (20-40)	1.61±0.12	56.15±8.53	21.39±0.95
GroupB n=40	30.70±7.629^{NS} (20-40)	1.65±0.09^{NS}	63.06±8.08***	23.62±0.54***

Data were expressed as mean ± SD. Unpaired t test were done.
Group A: Apparently healthy subjects of normal weight with BMI 18.5- 22.9 kg/m2.
Group B: Apparently healthy subjects of overweight with BMI 23- 24.9 kg/m2.
n = Number of subjects.
P value <0.05 was taken as level of significance.
SD = Standard deviation.
NS = p > 0.05
***= p<0.001

The mean ± SD of resting heart rate were 68.6000 ± 3.30346 beats/min in group A and 70.8000 ± 4.89479 beats/min in group B. The mean resting heart rate were non-significant (p > 0.05) in group B than group A (Table – II).

Table -II: Showing mean ± SD of resting heart rate of the study subjects in group A and group B.

Variables	Group A n= 40	Group B n=40
Resting heart rate(beats/min)	68.60±3.30	70.80±4.89^{NS}

Data were expressed as mean ± SD. Unpaired t test were done.
Group A: Apparently healthy subjects of normal weight with BMI 18.5- 22.9 kg/m2.
Group B: Apparently healthy subjects of overweight with BMI 23- 24.9 kg/m2.
n = Number of subjects.
P value <0.05 was taken as level of significance.
SD = Standard deviation.
NS = p > 0.05
Normal Heart rate = 60 – 90 beats / min58.

The mean ± SD of valsalva ratio were 1.5010 ± .0.32360 & 1.3367 ± 0.20453 in group A & B respectively. The mean values were significantly (p < 0.05) lower in group B than group A (Table –III).

Table -III: Showing mean ± SD of valsalva ratio of the study subjects in group A and group B.

Variables	Group A n= 40	Group B n=40
valsalva ratio	1.50 ± 0.32	1.33 ± 0.204**

Data were expressed as mean ± SD. Unpaired t test were done.
Group A: Apparently healthy subjects of normal weight with BMI 18.5- 22.9 kg/m2.
Group B: Apparently healthy subjects of overweight with BMI 23- 24.9 kg/m2.
n = Number of subjects.
P value <0.05 was taken as level of significance.
SD = Standard deviation.
**= $p < 0.01$
Normal value:
Heart rate response to valsalva maneuver: ≥ 1.21

DISCUSSION

The present study was carried out to observe the changes in heart rate in response valsalva maneuver test between apparently healthy normal weight and overweight individuals. BMI was calculated to differentiate normal weight and overweight individual. In overweight individuals, heart rate response to valsalva maneuver was significantly ($p<0.05$) lower than normal weight individual. This finding is consistent with that of some other investigators[4, 16,17]. In contrast some other researchers did not find any significant difference in valsalva ratio in overweight and normal weight individuals[18,19].

The precise mechanism that leads to deterioration of cardiovagal function was not established clearly. Overweight is a state of reduced glucose threshold, insulin resistance and hyperinsulinaemia.

Gradual development of insulin resistance in target tissues with the beginning of excess weight gain in overweight and obesity is responsible for subsequent development of hyperinsulinaemia. This hyperinsulinaemia has got a role in low cardiac vagal activity. But several researchers made various suggestions such as high insulin level or insulin resistance may cause damage to autonomic nerves at any level of their reflex arc, insulin may cause deterioration of microcirculation in many tissues including nerves which may lead to neural ischemia and thereby damage of cardiac parasympathetic nerve terminals occur at the level of cardiac muscle or vascular wall5, 20. Parasympathetic damage and decrease vagal tone may also occur due to hyperinsulinaemia or insulin resistance or there may be decreased in baroreflex activity[21].

From this above discussion it may be concluded that significantly lower values of heart rate response to valsalva maneuver indicates parasympathetic impairment or decreased vagal tone may occur due to decreased baroreflex activity in overweight individuals. The parasympathetic influence is mediated by the stimulation of the vagus nerve and decreases heart rate.

CONCLUSION

In conclusion, the evaluation of overweight individuals by simple non invasive test, heart rate response to valsalva maneuver provide diagnostic information about early subclinical parsympathetic dysfunction and is a useful tool to monitor the efficacy of therapeutic and lifestyle modifications. As our study was cross-sectional and limited in sample size, we recommend studying this test in a larger population of different age groups for more accurately assess the overweight individuals to decrease non communicable disease burden in Bangladesh.

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Original article

Effects of Paddy Dusts on FVC, FEV1 and FEV1% in Female Rice Mill Workers without Protector.

Sara Jabeen Khandaker[1], Chandra Rani Sarkar[2], A. T. M Zoadur Rahim Zahid[3], Neaz Ahmed[4]

ABSTRACT:

BACKGROUND: *Among the global risk factors of morbidity and mortality around the world the occupational lung diseases are becoming a great concern. Exposure to paddy dusts in the rice mills causing the workers to be associated with a long history of proliferative and fibrotic diseases in the lungs and also adverse effects on skin, nose and eyes.* ***OBJECTIVE:*** *To assess the effects of paddy dusts on FVC, FEV_1 and FEV_1% of paddy dust exposed female rice mill workers.* ***METHODS:*** *This cross sectional study was conducted in the department of Physiology, Rangpur Medical College (RpMC), Rangpur from July, 2017 to June, 2018. The study group enrolled 30 healthy female rice mill workers without protector exposed to paddy dust during working in rice mills and the control consisted of age, sex, height, weight and BMI matched 30 healthy females from surrounding community who were not exposed to paddy dust. FVC, FEV1 and FEV_1% of all the subjects were measured by digital spirometer. Statistical analysis was done by independent sample 't' test.* ***RESULTS:*** *In this study the mean predicted values of FVC, FEV_1 and FEV_1% between the female rice mill workers without protector and control group were not significant. The mean measured value and percentage of predicted value of FVC and FEV_1 were significantly ($p< 0.05$) lower in the female rice mill workers without protector than the female control group but there were no statistically significant ($p> 0.05$) differences in mean measured value and percentage of predicted value of FEV_1% (FEV_1/FVC ratio).* ***CONCLUSION:*** *The mean measured value and percentage of predicted value of FVC and FEV_1 were significantly lower in female rice mill workers exposed to paddy dusts without protector indicate lung functions impairment.*

KEY WORDS: Paddy dust, FVC, FEV_1, FEV_1%.

INTRODUCTIOND

efinite type of occupational hazards are certainly related to occupational health, which studies on all factors relating to work, working methods, condition of work and the working environment[1]. Occupational respiratory disease can be defined as an acute or chronic disorder that arises from the inhalation of airborne agents in the work place mainly organic dust[2]. The dust exposed in rice mills is a complex organic dust with varied composition including particles of husk, cuticular hair, pollen, bacteria and mucous spore[3]. Paddy dust is known to have a silica content. The kind of exposure like paddy dust (containing biogenic silica) can

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cause both restrictive and obstructive type of lung diseases including such responses as extrinsic allergic alveolitis, toxic syndrome, farmer's lung, chronic bronchitis, asthma and interstitial lung diseases[4]. Decrease in various lung function parameters in paddy dust exposed workers may be due to exposure to industrial dust, poor ventilation, non use of face masks and lack of proper exhaust facility[1]. The occupational diseases depend on concentration, size, solubility, weight and shape of the dust particle along with the duration of exposure apart from variation in personal immunity[5]. The Swedish National Board of occupational safety and health has prescribed the dust exposure limit of 10 mg/m3 and 5 mg/m3 for normal and organic dust respectively[5]. Several reports have suggested unprotected dust in agricultural work stations may lead to pulmonary fibrosis exhibiting a significant drop in FEV_1 and FVC in the workers exposed to paddy dusts[6].

In occupational respiratory diseases, spirometry is one of the important diagnostic too[1]. Periodic testing by spirometer plays a significant role in detecting pulmonary diseases in its earlier stages when corrective measures are more likely to be beneficial[7]. To the best of researcher's knowledge there has not been any recent study to identify and assess the health risk of occupational health hazards and safety measures of the rice mill workers against paddy dust exposures in Bangladesh.

Thus the aim of this study was to identify and assess the occupational health hazards among paddy dust exposed workers by measuring this spirometric lung function parameters and recommended management protocols that would control and prevent these hazards from impacting on the health and wellbeing of the workers.

METHODS

This cross sectional analytical study was conducted in the department of Physiology in Rangpur Medical College from July 2017 to June 2018. Protocol of this study was approved by Ethical review committee of Rangpur Medical College. 30 apparently healthy non obese non smoker female rice mill workers without protector aged 20-45 years were selected as experimental (group-B) from different rice mills of different districts in Rangpur division. For this study Age, sex and BMI matched 30 apparently healthy non obese non smoker subjects(group-A) from surrounding community not exposed to paddy dust were selected as control.

Fasting serum glucose level, serum creatinine, serum alanine amino transferase levels were estimated in both the groups of the present study for exclusion of DM, kidney disease and liver disease. All the values of these parameters were within the normal range and thus all the subjects were and free from any type of chest deformities, asthma, COPD, DM, HTN kidney diseases and any type of systemic illness. Before performing lung function tests, all the subjects were briefed about the nature, purpose and benefits of the study. Informed written consent of the study subjects were taken in easily understandable Bengali phrases. Detailed family and medical history were taken. All the information were recorded in a prefixed data collection format. Pulmonary function test was done by Autospiro (Digital Spirometer). Data analysis was done by using a computer based softwere SPSS, 24.0 versions for window.

Statistical analysis was done by independent sample 't' test. P value <0.05 was accepted as level of significance.

RESULTS

Mean age, height, weight and body mass index were almost similar in female control and rice mill workers without protector and there were statistically no significant differences ($p>0.05$) between the groups.

Table 1: Mean(±SD) values of General characteristics of study subjects (n=60)

Parameters	GroupA(n=30)	Group B(n=30)
Age(years)	37.26±3.433	38.70±4.814
Height(m)	1.66±3.569	1.66±3.265
Weight(kg)	62.733±4.813	61.400±5.605
BMI(kg/m2)	22.484±1.416	22.016±1.822

In this study there was no significant difference of mean predicted values of FVC, FEV_1 and FEV_1% between group A and B. The mean measured value and percentage of predicted value of FVC and FEV_1 were significantly lower ($p< 0.05$) in group B than in group A.There were no statistically significant differences in mean measured value and percentage of predicted value of FEV_1/FVC ratio between group A (female control) and group B (female rice mill workers without protector).

Table 2: Showing statistical analysis of mean values of FVC, FEV_1 and FEV_1% of study subjects:

Lung function parameters	measurements	Mean(±SD)		't' value	P
		groupA	groupB		value
	Predicted value (L)	3.42 ± (.218)	3.38 ± (.220)	.730	.468^{NS}
FVC	Measured value (L)	3.22 ±(.306)	2.07 ± (.235)	17.958	0.000***
	Percentageofpredicted value(%)	98.00±(5.78)	61.26±(5.526)	25.137	0.000***
FEV_1	Predicted value (L)	2.96±(.186)	2.96 ±(.258)	-.103	.918^{NS}
	Measured value (L)	2.94±(.245)	1.73 ± (.164)	22.428	.000***
	Percentageofpredicted value(%)	100.20±(.58)	61.96±(.490)	40.594	.000***
FEV_1%	Predicted value (L)	87.03±(1.88)	86.36 ± (.764)	-1.796	.080^{NS}
	Measured value (L)	85.96±(2.04)	85.36 ± (1.73)	1.227	.225^{NS}
	Percentageofpredicted value (%)	99.56±(2.45)	98.86 ± (2.40)	1.115	.269^{NS}

DISCUSSION

In the present study, mean measured values of all lung function parameters in healthy control group were within normal range. There were no statistically significant differences of mean predicted values of FVC, FEV_1 and FEV_1% between the groups. The observations in this study indicated that although obstructive pulmonary impairment was more frequent, both types of changes were present in rice mill workers. Due to paddy dust exposure fall in FVC indicated restrictive lung changes and fall in FEV_1 indicated obstructive lung changes[8]. Both these decreases were statistically significant than the control subjects. Mean measured and percentage of predicted values of FVC of female rice mill workers without protector were significantly lower than the control females which findings were similar to the studies by other investigators also[3,4,6,8,9-11]. Some previous studies also showed significant decrease in mean measured and percentage of predicted values of FEV_1 in female rice mill workers without protector in comparison to the control as collaborated by this study[3,4,6,8,10-12]. No significant ($p>0.05$) change in FEV_1% between both the groups were found in this study which is further supported by similar findings observed by some other researchers[8,9,11]. The decrease in FVC might be due to much more changes to the bronchi and elastic components of lungs resulting in restrictive type of lung impairment. Decrease in FEV_1 showed that exposure to dust caused early obstructive pulmonary impairment which further increased with increase in numbers of years of exposure[13]. Though various suggestions made by different investigators as mentioned earlier as possible causes of lung functions impairment but it is difficult to comment on exact mechanisms in this type of study. From the discussions of researchers it covers a few way in this regard of mechanism; macrophages and mucocilliary system were responsible for the clearance of dust particles from the lungs. Inhalation of paddy dusts over long time might lower the ability of macrophages to eliminate particles and impaired the activity of cilia lining the bronchi with decreased rate of airway clearance. This might result in penetration of the dust particles into the interstitium. As a result of subsequent inflammatory responses, hypertrophy and hyperplasia of mucous glands occurred which were causing progressive impairment of lung functions with increased work of breathing resulting in lower values of FVC, FEV_1[14]. Another mechanism responsible for this inflammatory reaction was mentioned by the researchers supposed to be the chronic allegic effects of paddy dust which produced sufficient IgE in the body. IgE bound with the mast cell and basophil causing release of agents including SRSA, protease, histamine, neutrophil and eosinophil chemotactic factor at alveolar or bronchiolar site. Such type of immunologically induced inflammation due to inhalational exposure to a large variety of antigens caused increasing lung parenchymal and airway resistance secondary to tissue damages and inflammation mediated fibrotic changes[14]. In this study it is suggested that repeated microinsults to the lungs on each exposure to the toxic component of paddy dusts would have damaging effects, thus a long time exposure to paddy dusts cause progressive impairment of lung functions and adversely affects the parameters such as FVC, FEV_1. There is no effects on FEV_1/FVC ratio because may be due to proportionately decrease in FEV_1 and FVC in female experimental group without protector in comparison to the control subjects.

CONCLUSION

From this study , it can be concluded that rice mill workers without protector exposed to paddy dust usually suffer from impaired lung function. So the rice milling authority should implement appropriate protective measures like regular use of respirator, face masks, aprons, goggles, hand gloves. Making the use of these protectors should be mandatory during working, maintainance of proper ventilation, raising awareness about the occupational health hazards among the workers are essential for prevention of developing impaired lung functions.

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Original Article

Extension of the Lymphoid Follicles into Submucosa of PostmortemVermiform Appendix in Different Age Group of Bangladeshi People

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ABSTRACT

BACKGROUND: *The vermiform appendix is a worm-like, closed-ended, narrow, small tubular structure, projecting from posteromedial wall of cecum, having no known digestive function in human being for which it has been regarded as a vestigial remnant. The most characteristic histological feature of the appendix is the dense, nodular ring of lymphatic tissue in its wall which occupies the lamina propria and often pushes through the muscularis mucosae to invade the submucosa. This lymphoid tissue exists mostly in the form of lymphoid follicles. The lymphoid follicles of the vermiform appendix vary in respect to their number, diameter and location in different ages. Appendicitis is the most important clinical condition of the appendix which predominantly affects children. Many cases of acute appendicitis result from obstruction of the lumen of the appendix. In 60% of patients, obstruction is caused by hyperplasia of the intramural lymphoid follicles. This form of obstruction is mostly observed in children. The incidence of appendicitis coincides very well with the number, size and location of lymphoid follicles.* ***OBJECTIVES:*** *It is necessary to understand the histological structure of the vermiform appendix in order to correlate with the pathogenesis of appendicitis. In Bangladesh there is no adequate data regarding location of the lymphoid follicles of the vermiform appendix. The present study is aimed to observe the location and extension of the lymphoid follicles of the vermiform appendix in various age groups.* ***METHODS:*** *With this aim the present study was carried out in the department of Anatomy, Sir Salimullah Medical College, Dhaka, from January 2008 to June 2009. After taking approval from the Institutional Ethics Committee, sixty (60) postmortem vermiform appendices of different age groups collected by convenient sampling from unclaimed dead bodies of Bangladeshi cadavers which were under autopsy examination in the Department of Forensic Medicine of Dhaka Medical College (DMC) and Sir Salimullah Medical College (SSMC), Dhaka.* ***RESULTS:*** *In the present study, the highest percentage of the lymphoid follicles that extends into submucosa was 21.43±1.57 in group A (0-20 years), whereas the lowest percentage was 8.70±0.70 in group E (>50 years). The values were highly significant when group A, B, and C compared to E (P<0.001).* ***Conclusions:*** *In the present study, the percentage of extension of lymphoid follicles into submucosa gradually decreased with advancing age.*

KEY WORDS: vermiform appendix, lymphoid follicles, extension, Bangladeshi peoples

INTRODUCTION

The vermiform appendix is a small structure without any known important digestive function in the human being[1]. Currently, many workers regard it as a complex and highly specialized organ after considering its rich vascularity, histological differentiation and adaptive functions[2,3]. It is an integral part of the Gut Associated Lymphoid Tissue (GALT) system as the mucosa and submucosa are thickened and dominated by lymphoid follicles. Thus, the appendix functions as an

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organ of the immune system[4]. For years, the appendix was credited with very little physiological function. But recent views suggest that the appendix serves an important role in the fetus and in young adults.

Lymphoid tissue first appears in the appendix about 2 weeks after birth which is the time when the large bowel begins to be colonized with the necessary bacteria[4]. These follicles enlarge and increase steadily in number to a maximum of 200 at 15 to 20 years of age, and then decrease and practically disappear after the age of 60[5,6]. A thin layer of muscularis mucosa incompletely separates lamina propria from submucosa.

It is necessary to understand the histological structure of the vermiform appendix in order to correlate with the pathogenesis of appendicitis. There is no adequate data regarding location of the lymphoid follicles of the vermiform appendix in Bangladesh. The present study is aimed to observe the location and extension of the lymphoid follicles of the vermiform appendix in various age groups.

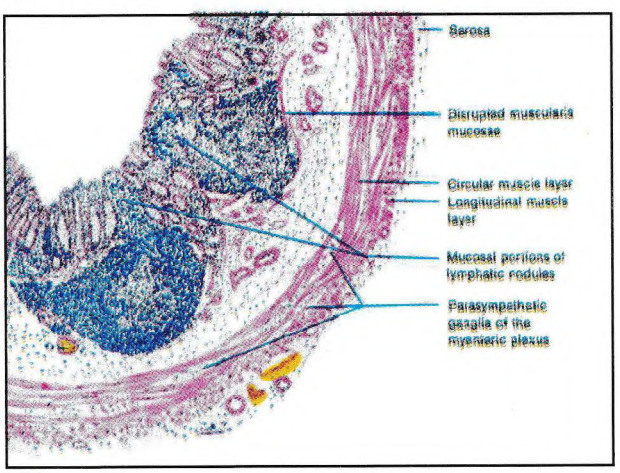


Figure 1: A low power enlarged view of a histological section of the vermiform appendix showing disrupted muscularis mucosae and submucosal extension of the lymphoid follicle

MATERIALS AND METHODS

The present study was descriptive with some analytical components, carried out in the department of Anatomy, Sir Salimullah Medical College, Dhaka, from January 2008 to June 2009, after approval of the protocol of the research by the Institutional Ethics Committee, on sixty (60) postmortem vermiform appendices of different age groups of Bangladeshi people. Samples of vermiform appendices with surrounding structures were collected by convenient sampling within 12 to 36 hours of death from unclaimed human dead bodies that showed no sign of putrefaction and that were autopsied on the different dates in the morgues of the Departments of Forensic Medicine of Dhaka Medical College (DMC) and Sir Salimullah Medical College (SSMC), Dhaka. The vermiform appendix was preserved in 10% formol saline. Initially, the collected samples were divided into five groups; Group-A (0-20 years), Group-B (21-30 years), Group-C (31-40 years), Group-D (41-50 years) and Group-E (>50 years). From each age group, six (6) fresh samples were selected for histological
study (Table 1).

Table 1: Age grouping of the vermiform appendices

Groups	Age in years	Total no. of samples (n=60)	No. of selected samples for histological study
A	0-20	9	6
B	21-30	19	6
C	31-40	12	6
D	41-50	14	6
E	>50	6	6

Three pieces of tissues were taken from the base, middle part and near the tip of the each appendix. One paraffin block was prepared with each piece of tissue. Then, several transverse sections were prepared for each region of each of the six appendices of each group. The sections were processed following standard histological procedures and were stained with routine hematoxylin and eosin stain. One good slide prepared as above from each tissue block was chosen for the study. As three slides were chosen from three regions of the same appendix, therefore, 3 x 6 = 18 slides were made for histological study from each group. Accordingly, 5 x 18 = 90 slides were prepared from five age groups. For the estimation of the percentage of lymphoid follicles which extend into submucosa, the entire section of each slide was focused under low magnification (X10) of microscope.

Estimation of the percentage of lymphoid follicles which extend into submucosa

At first total number of the lymphoid follicle is determined. It was done by using a circular transparent plastic sheet on which there was a computer-generated photographically produced line drawn at 12 o'clock position. The sheet was fixed on the top of the cover slip by adhesive tape. After that, by moving the stage of the microscope in a clockwise direction, the numbers of lymphoid follicles of the particular region (base, middle or tip) as well as the number of lymphoid follicles extending into the submucosa
of the specific vermiform appendix were counted and expressed in 'number of lymphoid follicles per low power field' and 'number of lymphoid follicles extending into submucosa per low power field' respectively (Figure 1). The mean value (±SD) of the six slides of each group was then calculated for each region. Two averages were calculated for each group. These are 'the average of the mean number of lymphoid follicles' and 'the average of the mean number of lymphoid follicles which extend into submucosa' at the three regions. Then the percentage of lymphoid follicles extending into submucosa in each group was calculated by the following formula:

Percentage of lymphoid follicles which extend into submucosa in each group =

$$\frac{\text{The average of the mean number of lymphoid follicles extending into submucosa}}{\text{The average of the mean number of lymphoid follicles}} \times 100$$

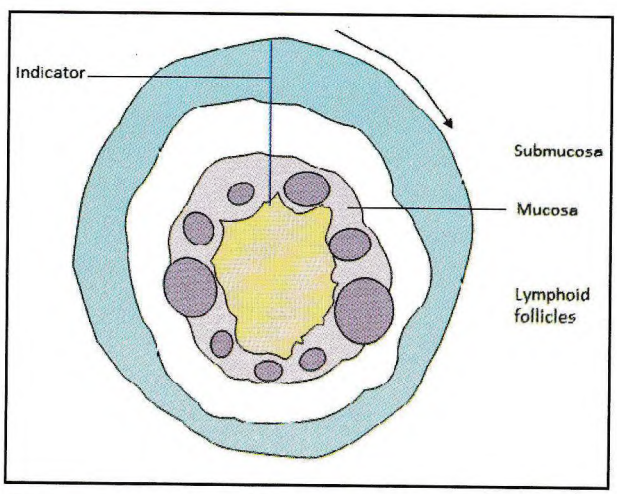


Figure 2: Procedure for counting the number of the lymphoid follicle and percentage of extension into submucosa

RESULTS

Table 2 and Fig. 3 show that the percentage frequencies of lymphoid follicle that extends into submucosa of vermiform appendices were 21.43±1.57 % in group A (0-20 years), 19.13±1.55 % in group B (21-30 years),

15.38±1.08 % in group C (31-40 years), 13.33±1.20 % in group D (41-50 years), and 8.70±0.70 % in group E (>50 years). Lymphoid follicle extending into submucosa was highly significant between group A and E, B and E, C and E (P<0.001), only significant between A and D, D and E (P<0.05) and between others were not significant.

Table 2: Percentage frequencies of the lymphoid follicles that extends into submucosa in different study groups

Groups	Age in years	Percentage of extension (Mean ± SD)
A	0-20	21.43±1.57 (25.00-17.39)
B	21-30	19.13±1.55 (15.38-22.22)
C	31-40	15.38±1.08 (13.04-19.05)
D	41-50	13.33±1.20 (10.00-17.65)
E	>50	8.70±0.70 (0.00-12.50)

Table 3: Comparison of percentage frequencies of the lymphoid follicles that extends into submucosa between different age groups

Groups compared	Significance of difference (p)
A vs. B	$>0.05^{NS}$
A vs. C	$>0.05^{NS}$
A vs. D	<0.05*
A vs. E	<0.001***
B vs. C	$>0.05^{NS}$
B vs. D	$>0.05^{NS}$
B vs. E	<0.001***
C vs. D	>0.05
C vs. E	<0.001***
D vs. E	<0.05*

Figures in the parentheses indicate range. Statistical analysis was done by ANOVA (multiple comparison), ns = not significant, ** = very significant, * = highly significant.**

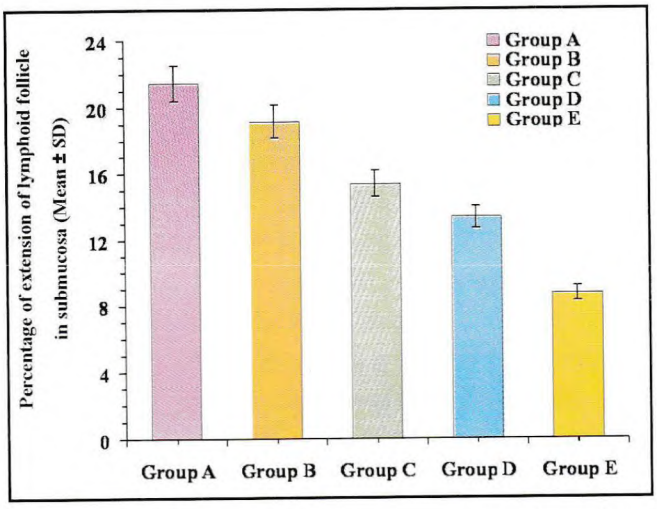


Figure 3: Lymphoid follicle extending into submucosa in different study groups

DISCUSSION

In the present study, the highest percentage of the lymphoid follicles that extends into submucosa was 21.43±1.57 in group A (0-20 years), whereas the lowest percentage was 8.70±0.70 in group E (>50 years). The values were highly significant when group A, B, and C compared to E (P<0.001).

From the above finding it could be stated that extension of lymphoid follicle into the submucosa gradually decreases with the increase of age or the size of lymphoid follicles are reduced with advancing age.

The finding of the present study consistent with Borley[2] (2005), Eroschenko[7] (2005), Ross et al[8] (1995), Young and Heath[9] (2000), and Paul[10] (2007).

Then it was found that there were similarities regarding the extension of lymphoid follicle into sub mucosa with increase of age in western country. As the number & size of lymphatic nodules are reduced with advancing age it could be stated that function of appendix as an organ of immune system gradually losses its importance with advancing age. There should be more study to confirm this.

CONCLUSION AND SUGGESTIONS

In the present study, the percentage of extension of lymphoid follicles into submucosa gradually decreased with advancing age. Similar study with larger sample size in different age groups including different socioeconomic status is recommended. Comparative study about the gross and histomorphological characteristics of the vermiform appendix in various primates can be done. A developmental study can be undertaken to find out development of the mucosa and differentiation of the lymphocytes of the vermiform appendix of the human fetus.

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Original Article

Frequency distribution of ABO and rhesus blood groups in blood donors of a Tertiary Care Private Medical College Hospital

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ABSTRACT:

BACKGROUND: *ABO and Rhesus (Rh) blood group antigens are hereditary characters and are useful in population genetic studies, in resolving medico-legal issues and more importantly for the immunological safety of blood during transfusion.* ***OBJECTIVES:*** *To study the pattern and frequency of blood transfusion as per ABO and Rh blood group system in a tertiary level private medical College. Study can help to prepare an inventory of highest frequency blood group containing regional population. That may facilitate and quicken the transfusion procedure in an emergency.* ***METHODS:*** *This retrospective study was conducted on the basis of blood transfusion register and relevant documents preserved in blood bank of Prime Medical College Hospital, Rangpur.* ***RESULTS:*** *The most common blood group was O (33.37%) and least common being AB (10.30%). Blood group A and B had almost same frequency. The prevalence of Rhesus positive and negative distribution in the studied population was 97.80% and 2.20% respectively.* ***CONCLUSION:*** *The study was conducted on 1660 blood donors. ABO and Rh typing was done using slide agglutination method with antisera ABO and Rh. The age group and sex of donors, frequency of ABO and Rh blood groups were reported in simple percentages. Male donors were more than female donors. Blood group formula with respect to ABO blood group was found to be shown by formula O>B>A>AB. Knowledge of frequencies of the different blood groups is very important for blood banks and transfusion service policies that could contribute significantly to the National Health System.*

KEY WARDS: Blood group frequency, blood donors.

INTRODUCTION:

Erythrocytes contain on their surface a series of glycoproteins and glycolipids, which constitute agglutinogens. Development of these agglutinogens are genetically determined and appears early in foetal life and remain unchanged throughout the life[1]. The ABO blood group system was the first human blood group system that was discovered by Landsteiner in 1901[2]. Later in 1941 Landsteiner and Wiener defined the Rh blood group[3]. Together these two systems have proved to be the most important for the purposes of blood transfusion. Commonly considered blood group systems are ABO and Rh systems. The classical blood group system (ABO) is divided into four types on the basis

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of presence or absence of surface agglutinogens, A and B. Anti-A and Anti-B are naturally occurring antibodies present in the serum of persons lacking the corresponding antigen. These antibodies are capable of producing intravascular hemolysis in the case of incompatible blood transfusion[4].

D agglutinogen is highly antigenic and most significant. Rh negative (lacking D agglutinogen) persons produce Anti-D if they encounter the D agglutinogen through blood transfusion or pregnancy and causes haemolytic transfusion reaction or haemolytic disease of fetus and new born. For this reason, the Rh blood grouping is routinely determined in blood donors, blood recipients and in mothers[5].

Moreover, the ABO and Rh blood groups are useful in clinical studies, population genetic studies, researching population migration patterns as well as resolving certain medicolegal issues, particularly of disputed paternity cases[6].

All human populations have the same blood 8group systems; although they differ in the frequencies of different types. The incidence of ABO and Rh blood groups varies markedly in different races, ethnic groups and socioeconomic groups in different parts of the world[7]. This study was done to determine the frequency and distribution of ABO and Rh blood group pattern among the blood donors at a tertiary care private medical college hospital in Rangpur, Bangladesh. Information about the distribution of different blood group population at various levels is important for efficient blood bank delivery services.

This study was undertaken to fulfil the above requirement.

MATERIALS AND METHODS:

The present retrospective study was carried out at Prime Medical College Hospital blood bank, Rangpur. Due ethical clearance from the institutional ethical committee was taken prior to study. The data of present study corresponds to period of July 2017 to December 2018 and includes in- house voluntary and replacement donors. The donor has to fill up a registration form as a requisite which included the information like personal details, occupation and medical history. All the necessary examinations required for safe blood transfusion was done.

Persons possessing any abnormality in biochemical report were excluded from the study.

Particularly the haemoglobin content of donor less than 13g/dl was considered as exclusion criteria for the present study.

The donors were then examined by medical officer according to blood donor selection criteria for the purpose of screening.

Repeated blood donors as evident from the blood blank inventory was excluded from study.

Total 1660 donors were considered medically fit and were accepted for blood donation.

Before blood donation, ABO blood group and Rh typing was determined by slide agglutination method by using commercially available antiseras i.e. Anti-A, Anti-B and Anti-D. Data on frequency of ABO and Rh blood groups were reported in simple percentages.

OBSERVATIONS AND RESULTS:

According to personal details, all donors were inhabitants of the northern region of the country and mostly from the district of Rangpur.

Among the 1660 donors male donors was 1523(91.74%) and female donors was 137 (8.25%) (Table I).

Table I: distribution of subjects according to gender (n=1660).

Gender	Frequency of Donors No (%)
Male	1523 (91.75)
Female	137 (8.25)
Total=	1660 (100)

n= total number of subjects

Among the ABO blood group the most common blood group was O, 554 (33.37%) and least common being AB , 171 (10.30%). Blood group A and B had frequency close to each other, which were 442 (26.62%) & 493 (29.69%) respectively. In the Rh blood group, most of the donors were Rh positive, 1623(97.80%) than Rh negative, 37 (2.20%) (Table II).

Table I: distribution of subjects according to gender (n=1660).

ABO Blood group	No (%)
A	442 (26.62)
B	493 (29.69)
AB	171 (10.30)
O	554 (33.37)
Total	1660 (100)

n= total number of subjects

Table III: Distribution of Rh blood group among study population (n = 1660)

Rh Blood group	Frequency No (%)
Rh positive	1623 (97.80)
Rh negative	37 (2.20)
Total	1660 (100)

n= total number of subjects

DISCUSSION

In the present study the number of male donors was much higher in number than that of female donors. In countries like Bangladesh males are the principal bread earner of family in most of the cases although no such data are presently available. In our society females are consider morbidly more vulnerable; they also have to perform most of the house hold duties. That might be the cause of such a low female frequency. Less body weight, low haemoglobin concentration, less birth spacing may also cause them unfit as blood donor. This finding is in agreement with other studies also[8,9,10].

Frequency of ABO blood group in our study was O>B>A>AB. Varied frequency was found in other studies such as B>O>A>AB[8,9]. In a different study it was found as O>A>B>AB[10]. In all of the studies the least percentage was found for AB blood groups[8,9,10],which is consistent with our study. Again, in the present study, the number of O blood group person was highest which is similar to the results of a study[8].

In our study the majority of blood donors were Rh positive and very few were Rh negative. This result is similar to the results of some other studies but there is a little variation in percentage[8,9,10].

CONCLUSION:

The present study concludes that the most common blood group was O and least common was AB among the blood donors at Prime Medical College Hospital. Results of blood group A and B showed the nearer frequencies. Again most of the donors were having Rh positive blood than Rh negative. Blood donation by the females was very low in comparison to male donors and it can be increased by improving health status and

awareness regarding blood donation. As the data generated in the present study represents the frequency of ABO and Rh blood group systems in population of northern region of Bangladesh.

Study can help to prepare an inventory of highest frequency blood group containing regional population. That may facilitate and quicken the transfusion procedure in an emergency when urgent blood transfusion is required.

The present study may help to formulate an inventory of commonest blood group population in nearby area or region so that identification, detection and collection of required blood group be ease and successful.

Therefore it would be useful to improve blood bank service delivery in this area. Thus this study can also minimise the morbidity and mortality rate of the region.

and also contribute to improve national health indices.

Limitation and recommendation

The study was conducted following cluster sampling so to confirm the findings census or large scale survey should be done. It also does not reflect the distribution of blood group systems in other area of Bangladesh.

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Review Article

Age Dependent Neuroimmune Regulation Of Neuropathic Pain Sensitivity – A Narrative Review

Sayema Ainan[1], Md Abul Hasanat[2]

ABSTRACT

Neuropathic pain occurs as a result of any peripheral nerve injury or lesion. This can happen due to mechanical injury to the nerves themselves or by disease, such as in diabetic neuropathy. The body responds to nerve damage by initiating inflammation, the process by which the body attempts to remove the cause of injury and begin healing. Inflammation is thought to play a key role in both initiating and maintaining pain. There is now a convincing body of clue that, adults experience neuropathic pain, whereas infants do not. In adult, the nerve injury induces a pro-inflammatory response; in contrast, infant nerve damage results in anti-inflammatory responses which suppress the dorsal horn excitation. The concept has emerged that, by blocking this anti-inflammatory response in infant rodents following nerve damage, neuropathic pain can be induced. Different longitudinal studies show that, when rodents reach adolescence their neuroimmune profile switches from anti- to pro-inflammatory, corresponding with neuropathic pain onset. In this review attempt has been made to summarize the lines of evidences for neuropathic pain that occurs in adulthood but not infancy owing to an anti-inflammatory response that continues into childhood.

KEY WORDS: Neuroimmune, neuropathic pain, anti-inflammatory, pro-inflammatory, dorsal horn

INTRODUCTION

Neuropathic pain following peripheral nerve damage is observed in adulthood but not infancy, with the average median age of paediatric onset being 13 years in human[1]. It has been noted in the literature that nerve injury in infancy and childhood possibly could go unnoticed, but can appear in adolescence despite having no obvious origin (such as, functional pain)[2]. Peripheral nerve injury in primary life generates massive alterations in the neural circuitry that can modify spinal somatosensory function in the long run. And, these alterationsdepend precisely upon postnatal age that is the time when this nerve damage occurs[3].

Several researches using infant rat and mice models of neuropathic pain confirmed the near absence or suppressed allodynia (pain from stimuli that don't normally cause pain) and absence of pain hypersensitivity following nerve injury made before postnatal day 21[4,5,6,7]. Nonetheless, spared nerve injury (SNI), a standard partial denervation model8 produced significant mechanical allodynia with the threshold falling to 55% of control values at postnatal day 33[4]. This kind of delayed onset is not only restricted to mechanical allodynia but

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also includes other characteristic features of neuropathic pain, such as, cold allodynia and altered weight bearing[2].On the other hand, extensive mechanical allodynia, measured as a reduction in hindpaw cutaneous sensory threshold to 16% of control adult rats, within postoperative day 1 and persist for at least 28 days[4].

Similarly, clear allodynia is observed in adult rats of another model of juvenile nerve injury: chronic constriction injury (CCI) but no change in hindpaw sensitivity when performed at postnatal age 10[4]. These lab findings are consistent with clinical experiences. Melzack et al[9]. Observed considerable delay of average 7 years in development of phantom limb in children having earliest amputation[9].

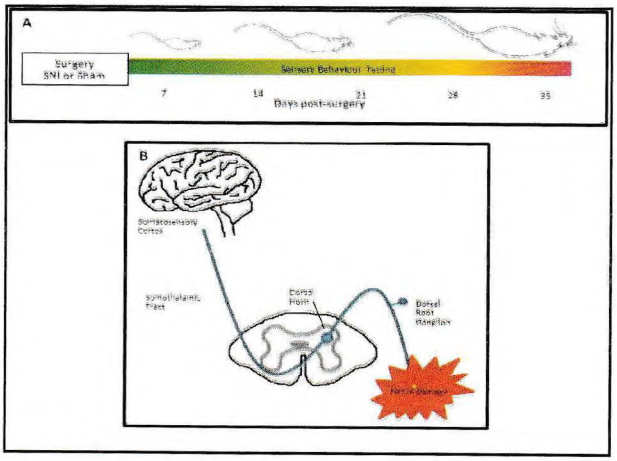


Figure 1 – A. Infant rodents exhibit an anti-inflammatory response to nerve damage, whereas adults exhibit a pro-inflammatory response. As the infant animals reach adolescence, the anti-inflammatory mediators are replaced by pro-inflammatory mediators and mechanical pain sensitivity emerges (Adapted from McKelvey et al.[2]). B. Neuropathic pain is transmitted via the dorsal horn of the spinal cord (Adapted from Crossman and Neary[10])

The body reacts to tissue injury by initiating inflammation – a process that recruits immune cells and release of inflammatory mediators, such as TNFα andBDNF[3]. This mechanism attempts to remove the cause of injury and start the healing process. Inflammation is therefore an important biological phenomenon, observed in various diseases and in response to the nerve damage seen in neuropathic pain[3]. Inflammation is thought to play a key role in both initiating and maintaining pain, though the mechanisms are not fully understood. Recently a group of researchers has highlighted the key role for pro- as well as antiinflammatory factors in the development and initiation of neuropathic pain by delineating the different inflammatory profiles seen in infants (who do not immediately experience pain) and adults (who do)[2]. According to recent longitudinal studies, adult neuropathic pain is supplemented with microglia activation, T-cell infiltration and the release of pro-inflammatory mediators in the dorsal horn, essential for sensitization and pain-like hypersensitivity[11,12]. However, this pro-inflammatory immune reaction is missing whenthe nerve injury is performed before postnatal age 21[5,6]. In addition, since infantrats and mice can develop clear pain hypersensitivityonly upon inflammation but without nerve damage[13,14] and also in response to glutamate or lipopolysaccharide application in spinal cord[5], the absence of neuropathic pain behaviour following nerve injury in infant rodents has been indicative as immature neuroimmune pathways, rather than inefficiency of pain circuitry itself[6,7].

This review would briefly outline the immediate and long term changes in spinal cord dorsal horn nociceptive circuits following experimental nerve injury in infant, juvenile and adolescent animal models, which will provide a clear insight about the delayed development of neuropathic pain that significantly, depends on maturation of neuroimmune regulation of pain pathways.

METHODS

Primary scientific literature on neuropathic pain was accessed as per journal articles keen to clinical studies and experimental models of neuropathic pain. Online databases were searched using a set of definite search terms and keywords including the ones mentioned in the current text. Databases searched included PubMed®, Google scholar®, and MEDLINE® resources. Special attention was given to screen and analyse information on the current concepts surrounding the maturation of neuroimmune regulation and pathogenesis of neuropathic pain pathways in the spinal cord dorsal horn as well as future research areas delineated in this field.

DISCUSSION

Pathophysiological alterations in pain processing over infancy, childhood and adolescence may be of special importance in comprehending development of neuropathic pain.In adults immune system plays a vital role for origin and development of neuropathic pain which is subjected to rapid onset and increase in the expression of pro-inflammatory mediators, including TNF, BDNF or IFN-γ results in sensitization of neurons in the dorsal horn and pain-like hypersensitivity[11,15,16]. On the other hand, absence of neuropathic pain following early life nerve injury has been attributed to the significantly lesser amount of glial response in the dorsal horn of the spinal cord. Spared nerve injury (SNI)performed at postnatal age 10 leads to little or not any increase in the expression of either microglia (MHC-II DMα, MHC-II DMβ, integrin-αM, CD68, IBA-1) or astrocyte (GFAP) markers in the dorsal horn spinal cord[5-7] and no increase the expression of pro-inflammatory mediators in the dorsal horn 7 days following surgery[2,6].

Likewise, the very weak microglial response to juvenile nerve injury is also accompanied by a remarkable increase in the expression of anti-inflammatory mediators, can be regarded as upregulation in the appearance of the transcription factor GATA[3], and the cytokines, IL-10 and IL-42 that differs noticeably from that following adult nerve injury. Until now, the origin of this upregulation remains unknown. GATA3 is a controller of T-cell development and stimulates the differentiation of CD4 T-cells into a Th2 cell lineage as well as promotes secretion of the anti-inflammatory cytokines: IL-4, IL-10, and IL-13 whereas inhibit production of IFN-γ and differentiation of Th1 cell[17,18]. IL-4 signalling is crucial for T-cell proliferation and differentiation into a Th2 phenotype and inhibitsthe expression of macrophage, microglia M1 phenotypes and proinflammatory mediators[19]. Also, IL-10 suppresses release of pro-inflammatory mediators and decreases the recruitment of glia cells in the spinal cord[20-22].

However, immature pain circuitry of infant mice is capable responding to proinflammatory mediators. Neonatal spinal dorsal horn lamina I neurons can be sensitized to TNF in vitro[23], besides intrathecal administration of TNF and lipopolysaccharide (LPS) can trigger microglia and in both condition mechanical hypersensitivity is induced as they overcome the anti-inflammatory activity following nerve injury[2]. Therefore infant spinal microglia is noticeably activated by peripheral inflammatory stimulation[7] but apparently do not produce TNF and BDNF following nerve damage because of nerve injury-induced anti-inflammatory response.

It has been suggested that this kind of immune response in neonates favouring anti-inflammatory reactionis necessary for the transition between a sterile environment in

utero and the outside world[24]. An anti-inflammatory immune response would prevent undue reactions to microbes, and has also been hypothesised to facilitate the pruning of faulty synapses and the elimination of damaged cell debris in the process of normal development[24-26].

Interestingly, McKelveyet al[2]. Recently have provided an important new insight into 'unmasking' of neuropathic pain following SNI in infant mice by suppressing the anti-inflammatory reaction.These researchers showed a significant mechanical hypersensitivity after blocking the IL-10 activity with intrathecal functional blocking antibody at postnatal age 10. But, in adults, intrathecal administration of anti-IL-10 after SNI has no significant effect on mechanical thresholds at any time[2]. The release of IL-10 following nerve injury is functionally essential for the lack of mechanical hypersensitivity, this finding therefore implies that neuropathic pain is not absent in infants, but that it is actively suppressed and can be revealed by blocking the anti-inflammatory response.

In addition, in adult neuropathic models, exogenously administered IL-10 and IL- 4 exert anti-nociceptive action by suppressing pro-inflammatory cytokines, microglia responses and pain behaviour[22] thus far, in infants this antinociception acts naturally following nerve injury through anti-inflammatory reaction[2].

During the periods of early development microglia which are the leading innate immune cells in the central nervous system (CNS), play vital roles in synaptic pruning, developmental neuronal apoptosis and remodelling[27,28]. These resident immune cells are highly active and exhibits profound phagocytic activity during the postnatal period in comparison to adult microglia withlittle mitotic activity and enormous motile processes of, which is critical for maintenance of microenvironment[29].

It is well documented in recent longitudinal studies that the immune profile in both peripheral T-cells and central resident immune cells microgliago through a developmental shift with age from an anti-inflammatory response (such as high IL-10) to pro-inflammatory in both rodents and humans[2,24,30]. For instance, Scheffel et al.[31], in their cytokine analysis showeda gradual age-related developmental shift of LPS-induced microglia between postnatal age 0 to 49 mice with an increasing expression of TNF-α, IL-1β, IL-6 and a reduced arginase[31].

Besides, studies in the healthy brain show indispensible roles for microglia during postnatal development which includes not only removal of cellular debris and dead cells via phagocytosis but also to establish synaptic contacts and control the size of dendritic spines when needed[28,32]. Since critical reorganization and modification of synaptic connections occur in the dorsal horn in the early weeks[33], it is likely that dorsal horn microglia are aiding these reorganization and modification of synaptic connections and pruning of axonal projections.Thus it can be proposed that, the anti-inflammatory response in the dorsal horn following nerve injury in infants may be the incidental consequence of the requirements for normal postnatal development[34,35,36]. So, a peripheral nerve injury (at postnatal age 10) can trigger, a default anti-inflammatory immune response in the dorsal horn spinal cord, responsible for absence of pain like behaviour, yet letting normal postnatal development to occur through phagocytic activity, without enabling detrimental inflammatory response and accompanying hypersensitivity[2].

Nonetheless, as the animal grows up towards adolescence, the neuroimmune regulation progressively transforms towards a pro-inflammatory reaction, which provide defence against foreign invasion, also allows nociception, neuronal sensitization and nociceptive behaviour.

The finding that delayed-onset pain hypersensitivity arises at adolescence in animal models is consistent with the observation that infant phantom limb pain does not appear for many years following the loss of limbs during infancy, in certain cases up until 3 to 15 years later[9]. An injury unalleviated in early life might have consequences that are suppressed by an anti-inflammatory immune reaction at first but the alteration in the balance of anti-inflammatory to proinflammatory activity in adolescence uncover the latent neuropathic pain hypersensitivity.

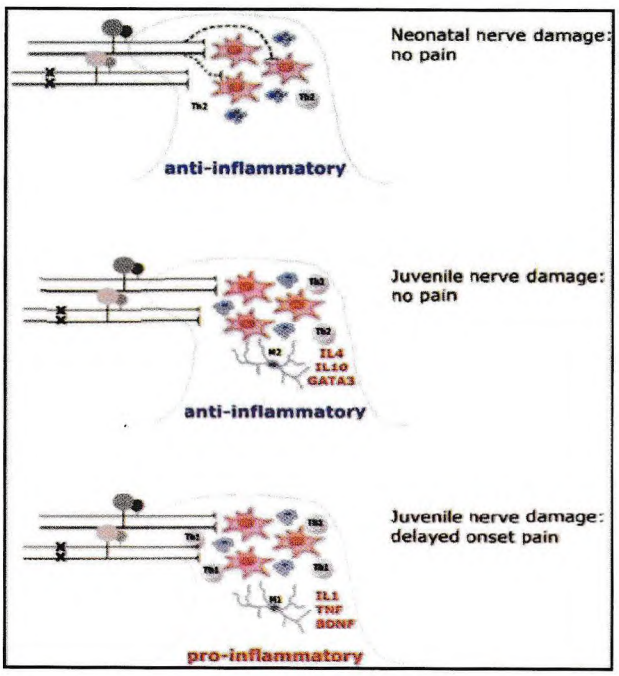


Figure 2.A potential model of the cellular activity in the dorsal horn following nerve damage at different time points and postnatal stages Top: Neonatal (day 1–7) nerve injury leads to primary afferent sprouting, and there is absence of pain behaviour. It is suggested that microglia (blue cells) are phagocytic and resident T helper cells (pink circular cells) are predominantly type Th2, which is anti-inflammatory. This cellular environment promotes and supports structural plasticity and postnatal neuronal circuit development (dark red neurons). Centre: Juvenile (day 10) nerve injury at first elicits an anti-inflammatory response in thedorsal horn with anti-inflammatory Thelper cell activity (Th2) and microglial activity (M2) along with release of cytokines such as IL4 and IL10. There is no pain behaviour. Bottom: Juvenile (day 10) nerve injury results in delayed onset of pro-inflammatory response in the dorsal horn, which possibly arises from a shift of microglial and T cell activity from M2 and Th2 to M1 and Th1, related to release of proinflammatory cytokines such as IL1, TNF and the neurotrophin, BDNF. These, in turn, stimulate dorsal horn neurons and as a result generate pain behaviour. (Adapted from Fitzgerald and McKelvey[3])

CONCLUSION

Though it is beyond the scope to review all the plausible mechanism, we have attempted to summarize the noteworthy data and observations which have significant relevance to the understanding of neuropathic pain. Following nerve injury in infant, the anti-inflammatory mechanisms suppress the sensitization of in the spinal cord dorsal horn that results in natural inhibition of pain. But as animal matures towards adolescence, neuroimmune regulation changes towards proinflammatory process responsible for neuronal sensitization that causes development of pain hypersensitivity.

The substantial role played by anti-inflammatory mediators has potential for a therapeutic solution to chronic neuropathic pain. However, questions are also raised regarding a possible role for both microglia and damaged C-fibre terminals.

Further investigation into the origin of the adolescent pro-inflammatory response is warranted and may provide greater understanding of neuropathic pain mechanisms as well as further therapeutic targets.

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