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Original Research Paper

International Physiology
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Effect of *Pseudomonas Aeruginosa* Extract on Human Blood Cells

Vijay Mane[1], Satish Dipankar[2], Nitin Goel Insan[3], A.D. Urhekar[4]

Abstract

This study was conducted to observe the effect of *Pseudomonas aeruginosa* extract on different human blood cells with different interval of time. Venous blood was collected from laboratory personnel in EDTA tube. *Pseudomonas aeruginosa* was isolated from different clinical samples. 20 different isolates were cultured on fresh nutrient agar after 24 hours of incubation at 37^0C. Isolates were centrifuged. The bacteria were resuspended in cold Sterile Distilled Water (SDW) and disrupted by sonication on ice for three times after 1 min intervals. Extract filtered through 0.22 µm Millipore Millex. Extracts were incubated with human blood. After incubation time, blood was used to observe effect on extract, on cells in cell counter. This study shows the effect of *Pseudomonas aeruginosa* extract on human blood cells. In this study, 20 Non-duplicate isolates of Non-MDR, MDR and PDR Pseudomonas aeruginosa were tested. Blood Sample (Heparinized venous blood) was collected in EDTA tube from healthy personnel. Bacterial isolation and further processing of extraction of toxin was done as per the standard procedure. Significant difference of *Pseudomonas aeruginosa* extract of all three groups was seen on human blood cells. Effect of extract was same on blood cells at particular interval of time.

Keywords: *Pseudomonas Aeruginosa*; Extract; Venous Blood; Blood Cells; Toxin Effect.

Introduction

P. aeruginosa is a non-fermentative Gram negative bacteria widely distributed in nature and can survive on a wide variety of surfaces and in hospital environment [1].

Pseudomonas aeruginosa is known to cause a wide spectrum of diseases. It can infect almost any external site or any internal organ, and therefore can be isolated from various clinical samples such as pus, sputum, urine, blood etc [2,3].

Pseudomonas aeruginosa is a leading cause of nosocomial as well as wound infections such as burn wounds. It is responsible for 10% of all hospital-acquired infections. Infections caused by *P. aeruginosa* are often severe and life threatening and are difficult to treat because of the limited susceptibility to antimicrobial agents and the high frequency of an emergence of antibiotic resistance during therapy thus resulting in severe adverse outcomes [4,5].

Pseudomonas aeruginosa acquired resistance to readily conventional antimicrobials following intensive use of antimicrobials [6]. It can be categorized as Multi-drug resistant (MDR) and Pan-drug resistant (PDR).

Multi-drug resistance is defined as non-susceptibility to at least one agent in three or more antimicrobial categories [7].

Pan-drug resistance is defined as isolates intermediately-resistant or totally resistant to all antimicrobial agents available for clinical use according to routine disk diffusion susceptibility results [8,9]. The prefix "pan" has its origin in the ancient Greek language, meaning "all" or "whole" [10].

Toxigenesis or the ability to produce toxins is an underlying mechanism by which many bacterial pathogens produce disease [11]. Toxins are virulence determinants that play an important role in microbial pathogenicity and evasion of the host immune response. This makes them ideal targets for the development of novel antimicrobial strategies. The potential applications of toxin research extend beyond simply combating microbial pathogens and include use as novel anti-cancer drugs and other front-line medicines and as tools in neurobiology [12].

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Materials and Methods

Bacterial Extract Preparation [13]

Collection of Blood Sample

Heparinized (20 ml) venous blood was collected in EDTA tube from healthy personnel.

Procedure

The bacterial population isolated from aqueous extracts. The bacteria were removed from the culture medium by centrifugation at 10,000 rpm for 1 hour. The bacteria were further processed, using one of the following a procedure:

In this procedure, the bacteria were resuspended in cold Sterile Distilled Water (SDW) and disrupted by sonication on ice for three times after 1 min intervals. This was followed by centrifugation at 10,000 rpm for 1 hour and lyophilisation of the pellet and supernatant and finally filtered through 0.22 µm Millipore Millex.

Effect of Extract on Blood Cells

In this study, 10 Non-MDR, 08 MDR and 02 PAN-drug resistances of *Pseudomonas aeruginosa* (PSA) isolates were tested.

Procedure

1. Run 0.5ml blood samples (normal blood) in coulter counter from EDTA tube, considered as positive control.
2. Add 0.2 ml extract in 2 ml blood for MDR and Non-MDR PSA isolates and incubate at 37^0C for 1 hour.
3. Take first reading of the entire sample in coulter cell counter after 1hour incubation.
4. Incubate the entire sample for 2 hours at 37^0C.
5. Take second reading of entire sample.
6. Incubate the entire sample for 3 hours at 37^0C.
7. Take third reading of entire sample.

Results

Table 1: Descriptive statistical analysis of in vitro effect of Non-MDR *Pseudomonas aeruginosa* extract on human blood cells

Parameter	Control	At 1 Hour	At 2 Hour	At 3 Hour	P value
WBC Count	6.8	5.57(0.25)	4.5(0.28)	3.51(0.28)	< 0.001
Hb Content	14.8	12.2(0.29)	10.54(0.26)	9.24(0.18)	< 0.001
Platelet Count	15.4	13.15(0.09)	11.66(0.36)	10.18(0.2)	< 0.001

** Significant at 1 % level, Units: WBCs: $10^3/mm^3$, Hb: gms/dl, PLTs: $10^4/ mm^3$, MDR: Multi Drug Resistance

Table 2: Descriptive statistical analysis of in vitro effect of MDR *Pseudomonas aeruginosa* extract on human blood cells

Parameter	Control	At 1 Hour	At 2 Hour	At 3 Hour	P value
WBC Count	6.8	5.2(0.6)	4.2(0.47)	3.1(0.59)	< 0.001
Hb Content	14.8	12.31(0.28)	9.97(0.58)	8.65(0.48)	< 0.001
Platelet Count	15.4	12.45(0.6)	11.16(0.7)	9.47(0.46)	< 0.001

** Significant at 1 % level, Units: WBCs: $10^3/mm^3$, Hb: gms/dl, Platelets: $10^4/ mm^3$, MDR: Multi Drug Resistance

Table 3: In-vitro effect of PDR *Pseudomonas aeruginosa* extract on human blood cells

Parameter	Control	At 1 Hour	At 2 Hour	At 3 Hour	P value
WBC Count	6.8	5.65(0.15)	4(0.1)	2.9(0.1)	< 0.001
Hb Content	14.8	11.5(0.7)	9.9(0.8)	8.7(0.4)	< 0.001
Platelet Count	15.4	12.15(1.0)	10.95(0.85)	9.6(0.3)	< 0.001

** Significant at 1 % level, Units: WBCs: $10^3/mm^3$, Hb: gms/dl, Platelets: $10^4/ mm^3$, PDR: Pan-Drug Resistance

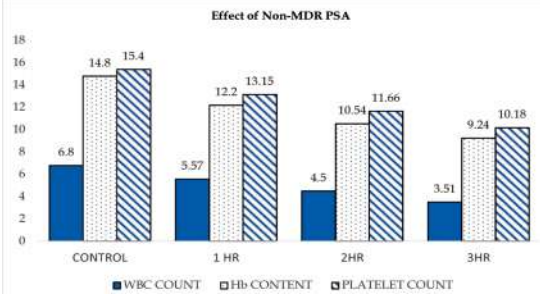

Fig. 1: In-vitro effect of Non-MDR *Pseudomonas aeruginosa* extract on human blood cells

Units: WBCs: $10^3/mm^3$, Hb: gms/dl, Platelets: $10^4/ mm^3$, MDR: Multidrug Resistant *Pseudomonas Aeruginosa.*

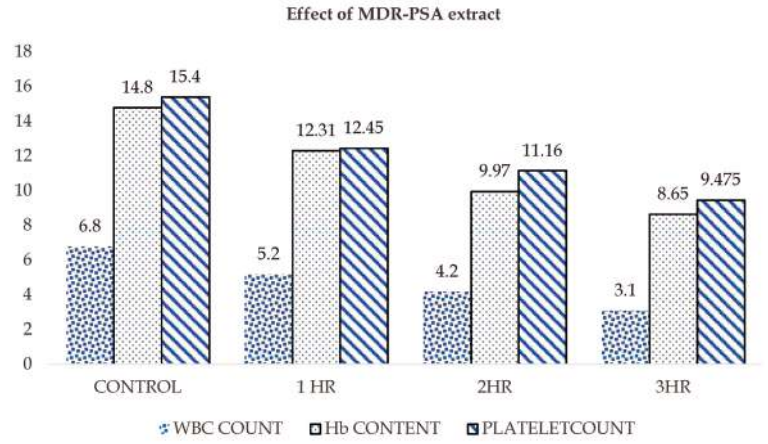

Fig. 2: In-vitro effect of MDR PSA extract on human blood cells

Units: WBCs: $10^3/mm^3$, Hb: gms/dl, Platelets: $10^4/ mm^3$, MDR, MDR: Multidrug Resistant

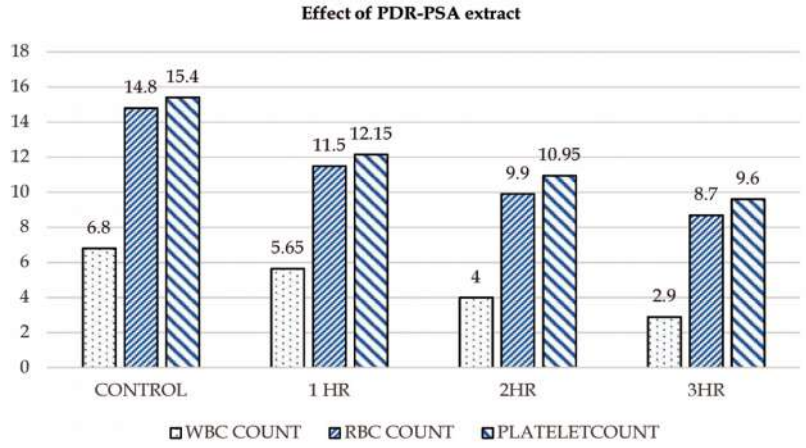

Fig. 3: In-vitro effect of PDR PSA extract on human blood cells

Units: WBCs: $10^3/mm^3$, Hb: gms/dl, Platelets: $10^4/ mm^3$, MDR, MDR: Multidrug Resistant

Discussion

Effect of Pseudomonas Aeruginosa Extract on Blood Cells

Aldona Baltec et. al. (1985), New York, studied effect of *Pseudomonas aeruginosa* cytotoxin on human serum and granulocyte. It was found that the lytic effect of *Pseudomonas aeruginosa* on Neutrophils was directly related to the concentration of cytotoxin and time of exposure [14].

Pseudomonas aeruginosa cytotoxin was found to agglutinate human erythrocytes in addition to its erythrocyte-agglutinating activity [15]. Therefore in present study we studied the effect of cytotoxin on hemoglobin content instead of effect cytotoxin of RBC count.

In the present study, effect of *Pseudomonas aeruginosa* extract showed rapid effect on blood cells in both MDR PSA and Non-MDR PSA.

Cell counts and hemoglobin content were decreasing steadily for interval of time in all three groups; MDR PSA, Non-MDR PSA and PDR which is statistically significant. This proves the cytolytic effect of *Pseudomonas aeruginosa* extract of blood cells.

Burn patients having bluish green discharge from their wounds is the most common and most serious life threatening infection; such patients require treatment with combination of higher antibiotics. In spite of such a massive treatment patients may not survive because of hemolytic effect of pseudomonas toxin.

Conclusion

Effect of extract of Non-MDRPA, MDRPA and PDRPA on human blood cells was studied.

Extract caused significant decrease in blood cells with respect to time interval.

Cytolytic effect of *Pseudomonas aeruginosa* extract contains cytotoxin produced by organism plays important role in pathogenesis of sepsis and opportunistic infections.

In spite of aseptic precautions; infection with Pseudomonas *aeruginosa* is frequently seen as this microorganism is highly resistant to antibiotics also mechanisms of pathogenesis are not clearly understood. Therefore this study suggest that along with combination of higher antibiotics whole blood transfusion should be given which will help the patient to withstand patient's immunity and mortality rate of patients infected with pseudomonas may decrease. This study also suggests need of further detail research to overcome the island of *Pseudomonas aeruginosa* resistance. Immunotherapy in human burns cases with antiserum to *Pseudomonas aeruginosa* may be useful. Further research study should be done to find out pathogenesis of infection caused by *Pseudomonas aeruginosa* also to find out *Pseudomonas aeruginosa* vaccines to combat this life threatening microorganism.

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Original Research Paper

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Correlation of Static Lung Function with Fat Free Mass & Fat Free Mass Index

Swikruti Behera[1], Bipin Bihari Pradhan[2]

Abstract

Static Lung Functions: besides physiological measurements, help in the diagnosis of underlying lung disease. It can be used to determine the severity of respiratory muscle involvement in neuromuscular disease, and can guide treatment decisions in several diseases too. This is a pioneering study to assess the correlation of fat free mass index(FFMI) with the static lung function instead of Body Mass Index(BMI). BMI can be misleading as it does not distinguish between the fat & muscle compartment. So, the aim of our study is to establish that FFMI should be used as reference variable for assessment of static lung functions rather than BMI.

Keywords: Static Lung Function; Fat Free Mass; Fat Free Mass Index; Body Mass Index.

Introduction

The belief, that weight shows little or no correlation with pulmonary function measurements [1], has been abandoned and Body Mass Index (BMI) is used as reference variable for various lung functions. Several studies have shown that elevated BMI is associated with impaired Pulmonary Function Parameters [2]. But BMI has limitation of not distinguishing between Body Fat & Body Fat Free Mass (FFM) [3]. Fat free mass includes muscle, bone, water & blood. Fat percentage is independent of stature and FFM resembles body mass as it is correlated with stature. The association is reduced or eliminated by expressing FFM as Fat Free Mass Index (FFMI).

$FFMI = FFM/Stature^2$

Measurement of static lung function, provide detail information regarding the functional status of lung & are determined by the elastic properties of lung. The result is used as an aid to diagnosis, for monitoring the progression of disease, or for the evaluation of therapy. Effect of obesity on respiratory function [2,4,5] has been established in various previous studies. This study is undertaken to assess if correlation of PFT exists with body fat percentage, FFM, FFMI and whether it is possible to establish them as lung reference variable.

Methods

The study was conducted on apparently healthy 150 medical students (85 males, 65 females) aged between 17 and 24 years. The experimental protocol was explained and written. Informed consent was obtained from all the volunteers. The Institutional Ethical Committee has approved the study, conducted between October 2010 and August, 2012. The subjects with history of smoking, asthma, any other past/ concurrent pulmonary diseases, and any other systemic diseases were excluded from the study. The study was conducted after 2 hours of light breakfast. To avoid circadian variation [6,7] all study were conducted between 10 am to 12 noon.

All anthropometric measurements such as age, sex, height and weight were recorded. Body weight was recorded in kilograms on empty bladder and wearing light weight clothing and bare foot with "Prestige Digital Weighing Scale". Standing height was recorded using "stadiometer" to the nearest 0.1cm. Waist circumference and Hip circumference were measured using measuring tape. Waist by hip ratio

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was calculated. BMI was calculated using Quetlet's Index[8]-BMI=Weight (in kg)/{Height(in meters)}^{2}

The body fat percentage was measured by "Bioelectric Impedance" analysis technique using 'OMRON Body Fat Monitor (HBF-306)'. Now FFM (100-Fat% × body weight) and FFMI (FFM/Ht^2) was calculated.

Pulmonary Function were recorded, on a window based "Flowhandy ZaN 100 USB & ZaN. GPI. 3xx", Germany, according to American Thoracic Society Guidelines [9].

Results

Observed finding are depicted in the Tables (1-4) and diagram (1-2).

Distribution According to Gender

Out of 150 subjects, 85 are males(57%) and 65 are females(43%) as depicted in Diagram → 1

Only BMI was found to be homogenous in both males and females. This is also depicted in the bar diagram in Diagram → 2.

Analysis was done using GraphPad Prism 6.0. Unpaired t-test, correlation and linear regression equation was used for the analysis.

The static lung values were found to be significantly different on Unpaired t-test.So, the male and female lung functions were compared separately with their respective body composition to avoid gender related variations.

Table 3 shows the various correlation coefficients of all the obesity markers like, BMI, BF%, Waist to hip ratio and muscularity or fitness markers like, FFM and FFMI and they are correlated with each static lung functions of males individually. This table shows that FFM followed by FFMI has highest correlation coefficients for all Static lung values except ERV which has highest significant negative correlation coefficient with BF%. BMI though significant but does not have highest correlation coefficient for any static lung function.

TV does not have significant relationship with any of the parameters.ERV has significant negative relationship with all body composition parameters except for FFM and W/H which does not have any significant correlation. ERV has highest negative correlation coefficient with BF% and BMI. IRV & IC has highest significant 'Pearson R' value for FFM followed by FFMI, Waist and Hip ratio, though all parameters have significant correlation with IRV and IC, but IC has insignificant correlation with W/H ratio. VC has significant positive correlation with FFM, whereas insignificant relation exists with all other parameters.

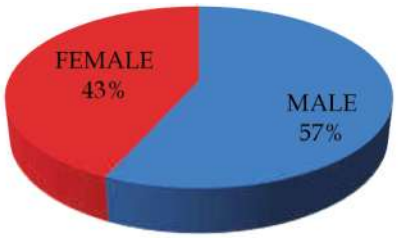

Diagram 1

Table 1: Anthropometric parameters (Mean ± SD)

	Males	Females
HEIGHT(In cm)	165.55 ± 5.8608	154.1158 ± 6.109
WEIGHT(In kg)	64.706 ± 11.7838	56.8153 ± 11.387
WAIST CIR(In inches)	31.846 ± 2.9081	30.118 ± 4.147
HIP CIR.(In inches)	36.984 ± 2.629	35.781 ± 3.853
WAIST/HIP RATIO	0.8606 ± 0.04819	0.841 ± 0.061
BMI (kg /m^2)	23.5108 ± 3.7207	23.9015 ± 4.4875
BF%	22.946 ± 5.1178	31.14 ± 6.125
FFM (kg)	49.4012 ± 6.7735	38.481 ± 4.816
FFMI (kg /m^2)	17.984 ± 1.9372	16.1866 ± 1.657

Diagram 2

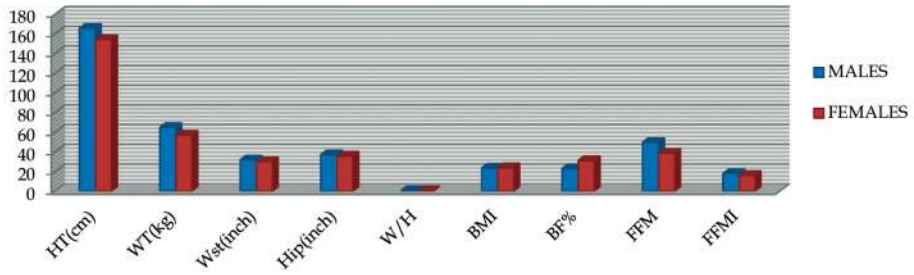

Table 2:

	Males	Females	p-value(Unpaired t)
ERV (In litres)	1.14837 ± 0.398798	0.833846 ± 0.311861	< 0.0001
IRV (In litres)	2.323587 ± 0.48605	1.691692 ± 0.357657	< 0.0001
TV (In litres)	0.578152 ± 0.291529	0.481231 ± 0.207539	< 0.0001
IC (In litres)	2.902065 ± 0.544876	2.172769 ± 0.370976	0.0236
VC (In litres)	4.047609 ± 0.528543	3.006769 ± 0.423142	< 0.0001

(P < 0.05→significant)

Table 3: Correlation coefficients/pearson R (males)

	BMI	BF%	FFM	FFMI	WHR
VC	0.2483	0.1144	0.5789	0.3289	0.2665
TV	0.2758	0.1830	0.3074	0.2412	0.06207
ERV	-0.4775	-0.4960	-0.1703	-0.3574	-0.1689
IC	0.6021	0.4853	0.6891	0.5884	0.3792
IRV	0.5131	0.4366	0.5913	0.5185	0.3889

(All the values in bold are significant values, i.e. p <0.05)

Table 4: Correlation coefficients (females)

	BMI	BF%	FFM	FFMI	W/H
VC	0.03225	0.04842	0.4491	0.06648	0.1034
TV	0.06460	0.1292	0.05422	0.002313	0.1449
ERV	-0.3698	-0.3697	-0.05877	-0.3109	-0.03205
IC	0.3477	0.3664	0.5614	0.3364	0.1449
IRV	0.3217	0.3040	0.5472	0.3464	0.07816

(All the figures in bold letters are Significant, i.e. p<0.05)

Discussion

Anthropometric Parameters: Table à1,Diagram à1&2 In our study on 150 subjects out of which 85 were males and 65(43%) were females. We have studied young healthy (17-24 years) subjects for the study. The number of volunteers studied was quite high compared to many workers. Lynell C & Phillip D (1995) [10] had conducted their study on 44 firefighters. Anuradha R et al, 2008 [11] studied on 132 males and females overweight students of age group 18-21 years. But here we have taken subjects of random stature irrespective of their BMI and body weight. Ceylan and co-workers, 2008 [12] studied only 53 volunteers. Lorenzo and co workers,2001 [13] studied on 30 obese adults.

Lorenzo and co workers, 2001 [13] and Anuradha R et al, 2008 [11] studied on obese adults. Nicholas S H and co-workers, 2007 [14] studied on 64 patients with stable COPD. Whereas we have studied healthy adults having BMI within normal range.

Mean Static Lung Function Tests

The male and female lung functions were compared separately with their respective body compositions to avoid gender related variations.Our views agree with the studies by Cotes et al [15], Hibert et al [16], Rosenthal et al [17] and Gibson et al [18].

Static Lung Function in Males

The correlation of ERV agrees with the findings ofAnuradha R et al 2008 [11], Ceylan and co-workers, 2008 [12] and Cotes et al (2001) [19]. They have discovered negative correlation of ERV with BF%. This proves the explanation that increased fat percentage in body leads to displacement of air by fat within thorax and abdomen [19].

But Contrary to the finding of Anuradha R et al 2008 [11], Ceylan and co-workers, 2008 [12] and Cotes et al (2001) [19] VC does not have negative correlation with BF%. Instead BF% does not have significant correlation with VC. But since VC can be increased with effort, it relates very significantly with Fat Free Mass (FFM) which coincides with the findings of Lorenzo and coworkers [13] and Ceylan and co-workers, 2008 [12] that loss of Fat Mass improves VC and Fat Free Mass has positive correlation with VC.

Waist and hip circumference showed positive correlation with all static parameters except ERV, which does not agree with the finding of Lorenzo and coworkers [13], Heather and co-workers, 1990 [20], Enzi et al,1990 [21] and Muls et al,1990 [22]. It may be because we have taken young adults with normal BMI contrary to obese subjects taken for study by the others.

FFMI had shown significant contribution towards IC as seen in study by Cotes et al, 2001 [19]. Our study shows the same but here FFM is found to have most

significant correlation factor followed by FFMI.

IRV and TV have not been studied much. Anuradha R et al, 2008 [11] had found no correlation with BF%. In our study we have also found TV has no significant correlation with BF%. But all these static lung values are effort dependent and their positive correlation with FFM proves the same.

Ray and co-workers, 1983 [4], Francoise and co-workers, 1993 [23] and Jones and Mary Magdalene, 2006 [2] observed that ERV and VC dropped significantly as BMI increased.

Static Lung Function in Females

Our study agrees with the study of Jones and Mary Magdalene, 2006 [2], Ray and co-workers, 1983 [4], Anuradha R et al, 2008 [11], andCeylan and co-workers, 2008 [12],where ERV had negative correlation with BF% and BMI , in females. But Ray and co-workers, 1983 [4] and Jones and Mary Magdalene, 2006 [2] observed that more increase in BMI will only lead to deterioration of lung functions like VC. W/H ratio was negatively correlated with static functions which is not the case in our study. This may be because we have not taken overtly obese girls for our study.

IC and IRV are not much studied in females, where we have found highly significant positive correlation with FFM which shows that, decline in fat free mass is associated with worsening of lung function [24].

Hence, neither BMI nor waist hip ratio can be considered as a reference variable. But BF% and Fat Free Mass (FFM) and Fat Free Mass Index (FFMI) can be considered as a consistent reference variable for static lung functions.

These observations show that changes in both fat and muscle can affect lungs; however, they can have opposite effect on VC and other indices so that when together considered as mass they can cancel each other out [19].

Conclusion

Static lung parameters have highest significant positive correlation with Fat Free Mass and Fat Free Mass Index, except ERV which has highest and significant negative correlation with Body Fat % in both males and females.

This study supports the view of the studies that respiratory muscle strength has effect on respiratory function [25,26]. As FFM & FFMI are direct assessment of muscle amount, so, increasing FFM by various exercises may prove to be useful in improving respiratory function rather than just losing weight.

Making allowances for body composition can improve the accuracy and biological relevance of reference equation for lung function [5]. The use of anthropometric and skinfold measurements has been criticised as being unreliable and inaccurate; they are unable to adequately assess adiposity and are liable to operator bias [27]. Limited usefulness of BMI should be taken into consideration and FFM & FFMI should be used as reference variable. Measurement of FFM by 'Bioelectrical Impedance' method is accurate, inexpensive, reliable, simple, safe and non-invasive technique for use in lung function laboratories [28, 29].

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Correlation Between Age, Gender and Brainstem Auditory Evoked Potential

Sunita Nighute[1], Maria Shaikh[2]

Abstract

Brainstem auditory evoked potential is a physiological technique for evaluation of auditory pathway. A number of electrical potentials can be recorded from the human scalp following acoustic stimulation. The potentials which occur within 10 msec of the stimulus onset termed the brain stem auditory evoked potentials (BAEPs). Latency appears to be the most stable measure and in consequence knowledge of the exact limits of normal latency of each wave is important. Since age and sex effects on central conduction time in the acoustic pathway are still debated, the following study was conducted to investigate possible age and sex differences in BAEP component latencies in younger and older male and females, total 60 of age 21-30yrs and 51-60 yrs respectively, The absolute peak latency of waves I, & V and interpeak latency of wave's I-III, & I-V in younger and older age group male and females are analyzed. The data was statistically compared between the different age groups and between the males and females and regression analysis was done. Absolute latencies of the waves I, and V and the interpeak latency of the waves, I-III and I-V showed significant increase with age, thus suggesting degenerative changes in the auditory pathway and synaptic delay. There were significantly increased values of the latencies of the waves I, and V and interpeak latencies of the waves, I-III, I-V in males as compared to the females. Thus, age and sex have an effect on latency and interpeak latency in Brainstem auditory evoked potentials.

Keywords: Auditory Evoked Potential; Interpeak Latency; Age and Sex.

Introduction

Evoked potentials provide a useful tool for neurophysiological research [1]. It is the record of electrical activity produced by groups of neurons within the spinal cord, brainstem, thalamus or cerebral hemispheres following stimulation of one or another specific system by means of visual, auditory, or somatosensory input. Brain stem auditory evoked potential (BAEP) recording is a physiological technique for evaluation of auditory pathway. BAEPs are the electrical activities resulting from the activation of the eighth nerve, cochlear nucleus, tracts and nuclei of the lateral lemniscuses and inferior colliculus [2]. These waves are generated at the following points of the auditory pathway: Wave I- Cochlear nerve, Wave II- Cochlear nuclei, Wave III- Superior olivary nucleus, Wave IV- Lateral leminiscus and Wave V- Inferior collicus. The clinical applications of BAEP consist of identification of neurological abnormalities in the VIIIth nerve & auditory pathways of brainstem and the estimation of hearing sensitivity. It is a measure of neural synchrony of the time-locked, on sensensitive, single-unit activity in the auditory nerve and the brainstem [3].

AEP is affected by factors like age, gender ,head size and hearing loss. The absolute peak latencies of the AEP waves increase with an increase in age. The waves I, III, and V have a direct influence on age [4]. Also, the interpeak latencies (IPLs) of the waves I-III,III-V and III-V in the older age groups had an increased value as compared to that in young people [5].

In humans, ABR can be recorded from about 26 weeks of gestational age (GA). After that, waves develop rapidly until term birth. From birth ABR

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continues development more slowly and in 18-24 month children, all the components are completely mature and adult-like [6,7]. In diagnostic audiology, interpeak latency interval (IPL) or inter-wave interval (IWI) of main ABR components especially I-V are very important because IPL I-V reflect the central conduction time (CCT) or brain stem conduction.

The influences of subject factors, especially advanced age, on the BAEP gain experimental attention. Fujikawa and Weber (1977), focusing on Wave V, found prolonged latency shifts from a 13 click/sec baseline response when older individuals were compared to young adults. Below the age of 2 years, interpeak latencies are prolonged relative to adult values. By the age of 2 years, the ranges for adults are reached, the absolute latencies of wave I, III, V increase by 0.1-0.2 msec with age. The reason for the age related latency shift is progressive myelination of the auditory tract. Some of the changes that occur in the aging auditory system may significantly influence the interpretation of the auditory brainstem responses in comparison with younger adults [8].

There also occurred gender differences of these waves. Males were found to have 0.1 to 0.2 ms longer latencies of the waves III and V and longer I–V interpeak intervals than females. The sources of the male and female related differences could be factors such as head size or gender-dependent sizes of the external acoustic meatus. Several factors may affect the peak latencies, IPL and wave amplitudes in ABR. These factors are classified as recording variables (electrodes, reference, filters), stimulus variables (stimulus intensity, stimulus rate, stimulus mode and stimulus phase) and subject variables (age, sex, body temperature, and cochlear hearing loss). Subject variables especially 'age' and 'gender' have powerful influences on ABR [5]. It has been shown that females may have shorter ABR latencies and IPL latencies than males. Also, in the elderly ABR waves have delayed latencies in comparison to young adults [9.10].

Since age and sex effects on central conduction time in the acoustic pathway are still debated, so the aim of our study is to investigate the differences, in BAEP component latencies in different age groups in male and females.

Materials and Method

In our study about sixty normal healthy subjects including both male and female in equal number were assigned to the following age groups:

2. 21-30 years (n=30)

5. 51-60 years (n=30)

BAEP test procedure was explained & written consent obtained from the subjects, a detailed history and thorough clinical & ENT examination were carried out to rule out any medical problem. Specific history was also taken to rule out any prolonged exposure to noise. Their height & weight were also taken.

BAEP recording was done in a quiet air conditioned room (28 + 1 oC). All the subjects were studied in the sitting position with appropriate head positioning so as to minimize postural muscle activity in the head & neck. The subjects were made to relax in order to minimize muscle artifacts. The recording surface electrodes filled with conductive paste were fixed on vertex (Cz, 10-20 international electrode placement system) & the on the mastoid process ipsilateral to the ear being stimulated. The ground electrode was placed on forehead (Fz). Electrodes were connected to the evoked potential recorder (RMS EMG. EP MARK II Machine manufactured by RMS recorder & medicare system, Chandigarh). Impedance of electrode was kept below 5 k ohms. A band pass of 100-3000 Hz was used to filter out undesirable frequencies in the surroundings. Responses to 2000 click presentation were averaged for 10 msec. Because of poor signal to noise ratio, it is necessary to average several hundreds of signal responses to get a recognizable BAEP waveform.

Brainstem Auditory Evoked Potential

The subject's hearing threshold was determined for each ear at the time of testing. The acoustic stimulus was rarefaction clicks, which were generated by passing 0.1 ms square pulses through shielded headphones. Clicks of intensity 60 dB above the hearing threshold were Delivered at the rate of 10 pulses per second .Monaural stimulation was used & contra lateral ear was masked by white noise at 30 dB below the click intensity. BAEP waves were identified & labeled. The peak latencies of waves I & V were measured. The interpeak latencies I-III, I-V was computed. Amplitudes of waves were also measured from peak to following trough of the wave. The waveform measured between the vertex and the ear be ear being stimulated constitutes the ipsilateral recording, whereas the waveform measured between the Vertex & ear opposite of the ear being stimulated constitutes the contra lateral recording.

Results

The mean and standard deviation of the absolute

peak latency and interpeak latency in male and female in milliseconds are shown in Table-1

The absolute peak latency of the waves I and V and the interpeak latencies of the waves, I-III and I-V were significantly increased in males than in females.

The mean & standard deviation of the absolute peak latency and interpeak latency in different age groups in milliseconds are shown in Table 2.

The data collected from both ears showed that increase in age will cause an increase in peak latency of wave I and V and the interpeak latencies of the waves, I-III and I-V were significantly increased in age from younger to older age.

Table 1: The mean and standard deviation of the absolute peak latency and interpeak latency in male and female in milliseconds.

Parameters	Males(30) Mean (Sd)	Female(30) Mean (Sd)	P-Value
Absolute Latency			
I	1.40±0.1	1.32±0.09	<0.001**
V	5.60±0.17	5.40±0.17	<0.001**
Interpeak Latency			
I-III	2.27±0.13	2.18±0.14	<0.001**
I-V	4.10±0.15	3.90±0.17	<0.001**

P-VALUE <0.001**= significant

Table 2: The mean & standard deviation of the absolute peak latency and interpeak latency in different age groups in milliseconds .

Parameters	AGE 21-30 yrs(30) Mean (SD)	AGE 51-60 yrs(30) Mean (SD)	P-Value
Absolute Latency			
I	1.75±0.049	1.85±0.035	<0.001**
V	5.50±0.022	5.70±0.019	<0.001**
Interpeak Latency			
I-III	2.02±0.021	2.09±0.148	<0.001**
I-V	3.29±0.077	3.90±0.038	<0.001**

P-VALUE <0.001**= significant

Discussion

The present study revealed that increase in age will cause an increase in peak latency and interpeak latency of waves I & V and interpeak latencies of the waves, I-III and I-V. There occurred significantly increased latencies of the waves I, and V and interpeak latencies of the waves, I-III and I-V in males as compared to the females, thus showing that age and gender affects these waves. Our study is comparable with the findings of previous one: Stephen W H (1981), observed peak latency increases in the elderly, to be due to peripheral processes [11]. Nai-Shin Chu (1985), showed small progressive prolongation in the peak latency with increasing age, particularly peak V [12]. Rosenhall U *et al* (1985), found latencies of waves I, III and V increase 0.1-0.2 msec with increasing age.

Harinder J S,earch.(2010) found positive correlation between the latencies of the waves III, IV and V and between the interpeak latencies of the waves I-III, III-V and I-V with age and sex in all the subjects [13].

Yones Lotfi, et all in October 2012 Their results indicate that there is a significant difference between males and females in absolute latencies and IPLs of ABR, irrespective of age. Females have shorter absolute latencies and IPLs in ABR. Furthermore, this study shows that absolute latencies and IPLs of ABR increase with aging especially in the 51-70 year-old interval [14].

It has been reported that females have shorter conduction times and ABR latencies than age matched males [15,9], and that gender has more powerful effects on ABR than aging [16-17]. Stuzebecher E et al (1987), which showed that the wave latency I and II and the interpeak latencies of the waves III-V showed statistically significant differences between males and females [18]. This finding is supported by Fallah TM (2007), showed increasing trend in age from younger to older caused values of interpeak latencies I-III , III-V & I-V increase 9. Rowe (1978) reported increased wave I-III interpeak latency in older than in young persons [19].

The increased latency and the interpeak latency which were observed in elderly individuals could be due to degenerative changes like auditory nerve

atrophy, synaptic delay and peripheral hearing loss with age. Increasing age also causes neuronal loss and changes in the permeability of the neural membrane, which might have led to the increased latencies of the BAEPs [20].

The latencies of waves III & V and interpeak latencies I-III and I-V are significantly higher in male as compared to female. Females have shorter interpeak latencies than males. This may be explained by shorter corresponding segments of the auditory pathway due to smaller brain size in female [21]. Aging changes that is, increases in latency attributable to increased conduction time in older subjects were observed in brainstem auditory pathway and males tended to show larger aging effects than females [22].

Our study is supported by Aoyagi M et al (1990) [9] & Harinder J S et al (2010) [13]. Aoyagi M et al (1990) investigated ABR latencies in 107 adults (57 males and 50 females) with normal hearing & found Wave III and wave V latencies and I-III and I-V interpeak latency intervals were significantly shorter in females than in males. He obtained significant positive correlations between head size and above-mentioned ABR wave latencies and IPLs. These results suggest that head size, which may reflect brain size, is one of the important factors for the basis of gender difference in ABR latencies. Harinder J S et al (2010) found BAEP waves III and V and interpeak latencies I-III and I-V are significantly higher in male as compared to female.

Conclusion

The results of this study among others show that age and gender affects the normal ABR latencies. and interpeak latencies this will be helpful for the interpretation and diagnosis in clinical practice.

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Effect of Lateral Positions of Body on Systemic Peripheral Resistance and Other Cardiovascular Parameters

Sharad Jain

Abstract

Lateral positions of the body have some effect on autonomic nervous system. Systemic peripheral resistance is the main determinant of after load to the heart. It is regulated by the arterioles via sympathetic nerves and directly affects the diastolic blood pressure. One hundred asymptomatic healthy male subjects, aged 17-23 years, participated voluntarily in the present study, undertaken, to assess the effect of lateral positions of body on systemic peripheral resistance and other cardiovascular parameters. Cardiovascular parameters were recorded by mercury sphygmomanometer and Impedance Cardiovasograph (Nivomon) in three positions supine, left lateral and right lateral decubitus. Results showed significant increase in systemic peripheral resistance, systemic peripheral resistance index, and diastolic blood pressure in left lateral position. All the parameters were significant decreased in right lateral decubitus position in comparison to supine. Systemic peripheral resistance was maximum in left lateral position which indicates maximum sympathetic activity in left lateral position among the three positions supine, left lateral and right lateral decubitus. So, one should avoid left lateral decubitus position during rest to avoid extra load on the heart.

Keywords: Systemic Peripheral Resistance; Impedance Cardiovasograph; Lateral Decubitus.

Introduction

All the cardiovascular parameters in human body are controlled by autonomic nervous system. Autonomic nervous system has sympathetic and parasympathetic limbs which work synergistically but usually in opposite ways. It is well known fact that when a person changes position from supine to standing, there is venous pooling of blood leading to decreased venous return which further leads to decreased stroke volume and cardiac output. As a result systolic blood pressure decreases until this is not compensated by decreased baroreceptors discharge due to less stretching of baroreceptors caused by reduced cardiac output which ultimately increase the sympathetic activity to increase heart rate and blood pressure until it returns normal. So it is evident that change in posture directly affects the autonomic nervous system. Many researchers have shown that postural stress in the form of head –up-tilt produces sustained increase in heart rate and rate pressure product and tilting can be used for assessing the integrity of autonomic cardiovascular regulatory mechanisms in physiological as well as clinical situations [1]. Few studies have shown the acute effects of varying degree of head down tilt in form of increase in cardiopulmonary blood volume, decrease in forearm vascular resistance but little change in BP and HR with 15° and 30° head down tilt and assumed them to be associated with a reflex decrease in muscle sympathetic nerve activity [2-3]. Studies have shown that angle & duration of tilt also affect the cardiovascular parameters as acute 30° head down tilt (HDT) did not produce significant changes in BP and HR while 60–80° HDT for 5 min resulted in a significant increase in diastolic blood pressure, decrease in pulse pressure and insignificant change in systolic pressure, heart rate and rate pressure product. They hypothesized that decrease in pulse pressure is most likely due to a fall in stroke volume due to excessive preloading of the ventricles while increase in Diastolic blood pressure could be due to sympathoexcitation due to raised intracranial

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tension occurring with 60° and 80° HDT [4]. Decreased vagal activity has been observed in in various physiological and pathological conditions such as congestive heart failure and coronary artery disease. In patients with CHF, the time for the right lateral decubitus position was two-fold longer than that for the supine and left lateral decubitus positions. The increased cardiac sympathetic activity and decreased vagal tone in CHF patients were normalized in the right lateral decubitus position and concluded that right lateral decubitus position in patients with CHF may be a self-protecting mechanism of attenuating the imbalance of cardiac autonomic nervous activity [5,6]. Autonomic effect of various recumbent positions, namely the supine, left lateral decubitus and right lateral decubitus positions, in healthy subjects by using spectral heart rate variability analysis has also been studied and suggested that cardiac vagal activity is greatest when the right lateral decubitus position is adopted [7]. All these studies suggest that change in posture of the body affects the autonomic nervous system. Change in heart rate, stroke volume and systemic peripheral resistance can directly detect more precisely the magnitude of change in autonomic status. Measurement of systemic peripheral resistance is most important among cardiovascular parameters especially in hypertensive patients and their siblings, because increase in systemic peripheral resistance is more harmful for heart as heart need to do more work for pumping the same amount of blood which may lead to ventricular hypertrophy leading to further need of more blood supply to the increased muscle mass in myocardium and further deterioration of the condition. Therefore any posture which may increase the systemic peripheral resistance should be avoided especially in hypertensive and pre hypertensive. Therefore the present study was conducted to find out the direct effect of change in posture of the body on systemic peripheral resistance and other cardiovascular parameters.

Material and Methods

The present study was conducted in the department of physiology, Saraswathi Institute of Medical Sciences, Hapur. One hundred asymptomatic healthy male subjects, aged 17-23 years, participated voluntarily in the present study, undertaken, to assess the effect of lateral positions of body on systemic peripheral resistance and other cardiovascular parameters. Experiment procedures were in accordance with the ethical committee on human experimentation. Study was carried out at ambient temperature with minimal external or internal sound disturbances in the room. Subjects reported to laboratory 2 hours after light lunch. They were explained in detail about the experimental procedure. Informed consent was taken from all subjects. Subjects were asked to lie in supine position. The color coded 8 leads of NICO patient cable were connected at their respective locations on the body of subjects. Blood pressure was recorded by using mercury sphygmomanometer. Systemic peripheral resistance, heart rate, cardiac output, and other parameters were recorded using Impedance Cardiovasograph (Nivomon). After 10 minute of rest in supine position, all the parameters were recorded. Then subjects changed position to left lateral decubitus. Parameters were recorded again after 10 minutes of rest in left lateral decubitus position. Finally subjects changed the position to right lateral decubitus. Parameters were recorded again after 10 minutes of rest in right lateral decubitus position. All data were collected and statistical analysis was done by One-Way ANOVA and Tukey post Hoc tests using the window SPSS Statistics 17.0 version.

Result

Table 1 shows that, in comparison to supine

Table 1: Comparison of cardiovascular parameters in supine left lateral decubitus and right lateral decubitus positions

S. N.		Supine	Left Lateral Decubitus	Right Lateral Decubitus
1	Systolic blood pressure (SBP) (mm Hg)	119.82±4.5	121.2±3.2	110.2±5.5##
2	Diastolic blood pressure (DBP) (mm Hg)	77.92±3.2	83.32±4.5*	72.12±4.1#
3	Heart rate (HR) (per minute)	70.80±1.13	72.1±2.8	67.18±0.23##
4	Cardiac Output (CO) (L/min)	5.41±0.2	5.62±0.17	5.02±0.28##
5	Stroke volume (SV) (ml/ beat)	74.04±0.82	76.28±2.5	70.24±0.26##
6	Systemic Peripheral Resistance (SPR) (dyne.sec/cm^5)	1360.1±17.4	1391.2±15.4*	1337.2±15.24#
7	Cardiac Index (CI) (L/min/m^2)	3.14±0.16	3.21±0.26	3.01±0.17##
8	Stroke volume Index (SI) (ml/ beat/m^2)	44.81±0.12	45.46±0.18	42.02±0.22##
9	Systemic Vascular Resistance Index (SVRI) (dyne.sec/cm^5/m^2)	777.5±11.5	791.5±13.1*	766.2±11.4#

Data presented are Mean±SD. *p<0.05, #p<0.05, ##p<0.01
*Comparison between parameters in supine ad left lateral decubitus
Comparison between parameters in supine ad right lateral decubitus

position, parameters in left lateral decubitus showed significant increase in systemic peripheral resistance, systemic peripheral resistance index, diastolic blood pressure ($p<0.05$) and insignificant increase in systolic blood pressure (SBP), heart rate (HR), cardiac output (CO), stroke volume (SV), cardiac index (CI) and stroke volume index (SI) ($p>0.05$). When compared to supine position, all the parameters in right lateral decubitus showed significant decrease. However decrease in parameters in right lateral decubitus was more significant in SBP, HR, CO, SV, CI, SI ($p<0.01$) and was less significant in DBP, SPR, SVRI ($p<0.05$).

Discussion

Blood pressure is regulated by autonomic nervous system. In hypertensive, sympathetic hyperactivity is one of the major causes of increase in blood pressure. Systemic peripheral resistance is mainly controlled by arterioles which have extensive sympathetic innervations. Sympathetic stimulation causes constriction of the arterioles and leads to increase in systemic peripheral resistance resulting in increase in diastolic blood pressure. Although systemic peripheral resistance also depend on other factors like temperature, viscosity of blood but these factors were remain unchanged in the present study. So it seems that change in autonomic activity is responsible for changes in parameters in the present study. Heart rate, stroke volume and systolic blood pressure decrease with decrease in sympathetic activity or increase in parasympathetic activity. Human S-A node receives its vagal innervations mainly from right vagus nerve. The right vagus nerve in the neck might be stimulated by periodic massage from the pulsation of the carotid artery in the right lateral decubitus position leading to higher parasympathetic activity. The position of the heart is lower in the left lateral decubitus than in the right lateral decubitus position. Gravity might exert an increased workload on cardiac function when the left lateral decubitus is assumed. A larger workload required in left lateral decubitus, as compared with the right lateral decubitus position, will produce more sympathetic and less vagal activity [8,9]. While reduction in this workload in right lateral decubitus position will lead to an enhancement of vagal activity. Because of right sided anatomical position of right atrium, the venous return from the venous system via inferior and superior vena cavae to the right atrium is more favorable when assuming the right lateral decubitus position, which may increase vagal activity. While in left lateral position, venous return is less in comparison to supine and right lateral decubitus. To compensate for decrease in venous return and cardiac output, sympathetic tone is enhanced and vagal tone is suppressed in left lateral position. Therefore there is possibility of, higher vagal activity and lower sympathetic activity in right lateral position with reversal of autonomic activity in left lateral position. Our results also indicate the increased parasympathetic activity in right lateral decubitus as shown by previous studies [10,11]. It can be also conclude from the present study that systemic peripheral resistance is significantly higher in left lateral decubitus so hypertensive as well as pre hypertensive people must avoid left lateral decubitus position during rest because it may be harmful for the heart while right lateral decubitus position should be preferred because it is associated with decreased peripheral resistance and increased parasympathetic activity.

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Original Research Paper

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Correlation between Body Mass Index (BMI), Skinfold Thickness and Speed and Power of Adolescent Cricket bowlers: A Cross Sectional Study Protocol

Vidushi Gupta[1], Vandana Esht[2], Asir John Samuel[2], Senthil P. Kumar[3]

Abstract

Background: The most exciting aspect in the game of Cricket is bowling. Adolescents shows increased involvement in sports due to which there is a risk and severity of sport injury. Few studies are there which correlate anthropometric characteristics and performance tests having age, BMI, skinfold thickness with power, speed tests in adolescent Cricket bowlers. *Objective*: To relate the BMI, Skinfold Thickness and speed and power of adolescent Cricket bowlers. *Methods:* A sample of 400 Bowlers will be recruited based on predetermined set of inclusion criteria from recognised school by multistage sampling method for cross-sectional study. Power and speed will be estimated by 6m timed hop test and 50-yard dash test respectively. *Stiatistical Analysis*: Results will be expressed as the mean ± standard deviation (SD). Data will be screened for normality using the Kolmogorv-Smirnov test and summarized using descriptive statistics. Pearson's correlation coefficient will be applied to establish the relationships among the variables measured. *Conclusion*: This study will help in determining the relationship between BMI, Skinfold thickness and speed and power in adolescents Cricket bowlers.

Keywords: Adolescent Cricket Bowlers; BMI; Skinfold Thickness; 6m Timed Hop Test; 50-Yard Dash Test.

Introduction

For most Indians, Cricket has been more than a game which serves as a fulcrum around which national identity is shaped. The game of the Cricket unites India more assuredly than any other thing [1]. The most exciting aspect in the game of Cricket is bowling [2]. During bowling there is a constant twisting, extension and rotation in a brief duration of time while body tissue and footwear absorb much ground reaction force [3]. Most of injuries in Cricket occurs while bowling. The bowlers of younger age are at big risk of injury, because of their incomplete growth process [4].

Adolescence is an age of human growth and maturation during which unique changes occur as well as many adult patterns are entrenched [5]. At an early age there is an increased involvement of adolescents in organized sports due to which there is a risk and severity of sport injury. Increased participation and training leads to more sports injuries. The leading causes of injury are sports and recreational injuries [6].

Gradual plan that respond and accommodates with injury or pathological issues and conditions is a feature of successful rehabilitation. To prevent further soft tissue injury, it involves adequate management of injury followed by progressive treatment protocol which is to be implemented effectively and key to successful implementation of progressive rehabilitation programme is goal planning [7].

For competitive athletes of all age groups pre-participation physical evaluation should take place. In case of adolescents, pre-participation physical evaluation determines general health, provide quality and cost effective health care [8]. For the evaluation of physical structure anthropometry is helpful and performance which is skill related is measured with specific tests [9,10].

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Anthropometry is defined as human body measurement in terms of dimensions of bone, muscle and adipose tissue. Presently selection of sportsman for higher performance is taking place on the grounds of physical structure and body size [11]. The bowlers are known to be lean and tall having little fat in their lower extrimites [12]. There was a strong positive relationship between high BMI and increased risk of injury as well as higher injury-related expenditure in adolescent age groups [13]. Measurements such as height, body weight, mid arm circumference and triceps skin fold thickness are commonly used for assessment [11]. In addition to technique of movement and experience, an important factor to athlete's performance may be the favourable anthropometric profile [14]. Performance testing allows menders to identify an athlete's strength as well as weakness. According to athlete's need, performance test enable them to accommodate and adjust training and rehabilitation [15].

Aim of the Study

To find out the relationship between BMI, Skinfold Thickness and speed and power of adolescents Cricket bowlers.

Objective of the Study

To relate the BMI, Skinfold Thickness and speed and power of adolescent Cricket bowlers

Review of Literature

Nuhmani and Akthar [16] characterize correlation between anthropometric traits and functional achievement of professional inferior tennis athletes of India. Hundred elite junior tennis players were assessed for anthropometric measurements including BMI, fat mass, calf circumference, thigh circumference, Waist- hip ratio, and muscle mass. Sergeant Chalk jump test, 40 yard sprint test and T test were used to assess performance. The findings revealed a positive interrelation between muscle group, circumference evaluation and functional achievement, and negative correlation between anthropometric characteristics like BMI, Waist Hip ratio and functional performance.

Lopes et al [17] studied association between BMI and motor abilities in children. Seven thousand one hundred seventy five students under the age of six to fourteen years were assessed. For evaluating motor abilities balance beam test, jumping laterally, hopping on one leg, shifting platforms were used. They found that motor abilities are inversely associated with BMI.

Nuhmani et al [18] evaluated Limb circumference and performance in junior tennis players. Hundred junior tennis players were assessed. Among Skinfold thickness calf and thigh circumference and for performance Sergeant Chalk jump test, 40 yard sprint test, T test were taken. They found a interrelation between both thigh and calf circumference and the entire three functional performance test.

Methodology

- Study Design: Cross-sectional study
- Study Setting: sports complex of respective school
- Sample Population: Adolescents
- Sampling Technique: Criteria based multistage sampling technique
- Sample Size: n=400
- Estimated by the Formula:

$$\frac{n= Z^2_{a/2}*P*(1-p)*D}{E^2}$$

Inclusion Criteria

- Adolescents (10-19year)
- Male and female gender
- Cricket bowlers

Exclusion Criteria

- Cricket batsman
- Musculoskeletal disorder
- Any cardiac or respiratory or neurological disorder
- Fracture and recent surgeries
- Who play sports other than Cricket

Variable

- Independent variable
  - ❖ Skin fold thickness
  - ❖ Weight
  - ❖ Height
- Dependent variable.
  - ❖ Conceptual dependent variables
    - ❍ Speed

- Power
- perational dependent variables
  - 50 yard Dash test
  - 6-m timed hop test

Instrumentation for Data Collection

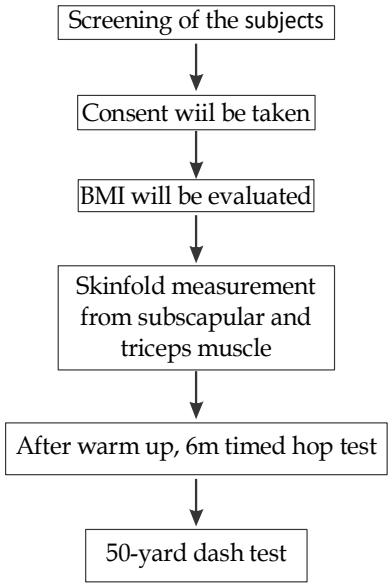
- Standard Measuring tape
- Skin fold calliper
- Weighing machine
- Stop watch

Outcome Measure

- Primary:
  - Skinfold thickness
  - Speed
- Secondary:
  - Height
  - Weight
  - Power

Protocol and Procedure

By taking written permission from principal of school, tester contact with the respective teacher for students who used to play Cricket. Screening will be done on the basis of selection criteria 400 adolescents who participate in Cricket as bowlers of age group 10-19 years will be recruited from schools of Mullana, Ambala district.



Procedure

Data Analysis

Results will be expressed as the mean ± standard deviation (SD). Data will be screened for normality using the Kolmogorv-Smirnov test and summarized using descriptive statistics. Pearson's correlation coefficient will be applied to establish the relationships among the variables measured. Data will be analyzed by using SPSS (Statistical Package for Social Science) version 16. A 5% error will be used to indicate statistical significance.

Discussion and Implication

This study will help in determining the relationship between BMI, Skinfold thickness and speed and power in adolescents Cricket bowlers. It will be helpful for the bowlers in their training program and rehabilitation. This study will be concluded in March 2016.

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Original Research Paper

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Early Morning May Not Be the Best Time of the Day to Study: Hypothesis

A.K. Singh[1], K.M. Shukla[2], P.K. Jain[3], S.K. Sant[4], S. Kumar[5], K. Jaiswal[6]

Abstract

Since childhood we have been advised by the to study or revise the assignments early morning. But the recent studies indicate that level of stress hormones is highest at the time of wakening. Stress hormones affect memory negatively. As the cortisol level is the highest in the morning just after awakening (in day workers) and because the excess of cortisol can impair the ability of the hippocampus to both encode and recall memories, thus it is hypothesised that morning time may not be the best time to study and revise assignments..

Keywords: Stress Hormones; Cortisol; Memory; Study Assignments.

Introduction

Since childhood we have been advised by the to study or revise the assignments early morning. But the recent studies indicate that level of stress hormones is highest at the time of wakening. Stress hormones affect memory negatively. In particular, the hippocampus, prefrontal cortex and the amygdala [1][2].

One class of stress hormone responsible for negatively affecting memory is the glucocorticoids (GCs), the most notable of which is cortisol [3][4][5]. Glucocorticoids facilitate and impair the actions of stress in the brain memory process[6]. Cortisol is a known biomarker for stress[7].

Under normal circumstances, the hippocampus regulates the production of cortisol through negative feedback because it has many receptors that are sensitive to these stress hormones. However, an excess of cortisol can impair the ability of the hippocampus to both encode and recall memories[4]. These stress hormones are also hindering the hippocampus from receiving enough energy by diverting glucose levels to surrounding muscle[4].

Hypothesis

As the cortisol level is the highest in the morning just after awakening (in day workers) and because the excess of cortisol can impair the ability of the hippocampus to both encode and recall memories, thus it is hypothesised that morning time may not be the best time to study and revise assignments.

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Review Paper

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Caffeine and Cognition: What do Event Related Potentials Say?

Abhinav Dixit

Abstract

Caffeine is a stimulant that is present in various drinks like tea, coffee and colas. It acts on the body via different mechanisms like altering intracellualar calcium, acting on benzodiazepine receptors and inhibition of phosphodiesterases. The main mechanism of action is blocking of adenosine receptors and alteration of the neurotransmitters in the brain.

The event related potentials are electrical signals in response to stimuli and represent the processing of information by the brain. Various studies have been done to evaluate the effect of caffeine on cognitive processes using event related potentials. This review summarizes the evidence of the effect of caffeine on cognition.

Keyword: Caffeine; Cognition; Evoked Potentials.

Introduction

Caffeine (1, 3, 7-trimethylxanthine) is one of the most widely used psychoactive drug in the world. It is consumed in various forms like tea, coffee, colas and many over the counter medicines. The amount of caffeine in food items ranges from 40-180 mg/150 ml of coffee, to 24-50 mg/150 ml of tea and 15-29 mg/180 ml for colas [1]. Caffeine is known to affect various body systems. In low doses of 50-250 mg per sitting, caffeine produces relaxation, increased alertness, feeling of well being and increased concentration. In doses ranging from 400-800 mg in one sitting, it causes tachycardia, nervousness, aggressiveness, insomnia, trebling and anxiety.

Caffeine is readily absorbed from oral, rectal and parenteral routes. The half life of caffeine is 3-7 hours [2]. Significant levels of caffeine are observed in brain within 5 minutes of oral intake with peak levels being observed within 30 minutes [3]. Metabolism of caffeine occurs mainly by demethylation to paraxanthine. A little amount of caffeine is also demethylated to form theobromine and theophylline [4].

Caffeine increases the amount of epinephrine and norepinephrine secreted by adrenal medulla leading to stimulation of respiratory centre with an increase in respiratory rate, oxygen consumption and carbon dioxide elimination. The cardiac muscle is directly stimulated by caffeine leading to increase in heart rate, cardiac output and force of contractions. Caffeine also causes stimulation of medullary vagal nuclei that in turn decreases the heart rate. Action of caffeine on smooth muscles of coronary, pulmonary and general systemic blood vessels causes them to dilate: at the same time stimulation of vasomotor centre causes vasoconstriction of these vessels [5].

Mechanism of Action of Caffeine

Various hypotheses have been formulated regarding the possible mechanisms of actions of caffeine at cellular level.

(i). One of the mechanisms of action of caffeine is mobilization of intracellular calcium. At a concentration of 1-2 mM caffeine decreases the excitability threshold and promotes translocation of calcium through plasma membrane and cytoplasmic reticulum [6]. However it has been found that mechanism occurs at doses higher than those attained by human consumption [1].

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(ii) Another mechanism which is proposed is Inhibition of phosphodiesterases by caffeine in the CNS [7].

(iii). Binding of caffeine to benzodiazepine receptors has also been observed [8]. It has been seen that caffeine antagonizes or modifies the effects of benzodiazepines on human behavior [9,10].

(iv). Via blocking of adenosine receptors: There is evidence that adenosine acts to decrease the rate of firing of neurons [11]. It has been observed that caffeine increased the amplitude of EPSP and population spike of hippocampal CA1 pyramidal cells. A reversible and concentration dependent antagonism of adenosine-evoked inhibition of EPSP was seen. The excitation reflected four changes in the neuronal membrane properties i.e. a depolarization from resting membrane potential, a decrease in membrane conductance, in long duration hyperpolarisation and that of accommodation [12].

The Adenosine receptors are present in almost all areas of brain with the maximum concentration being in hippocampus, cerebral and cerebellar cortex and certain thalamic nuclei [13]. Morgan et al reported that caffeine caused a dose dependent (30-75mg/Kg) increase in dopamine in the striatum [14]. Caffeine by blocking adenosine receptors thus brings about changes in the turnover of various neurotransmitters like adrenaline, dopamine, serotonin, acetylcholine, glutamate and GABA [15].

Evoked Potentials

Evoked potentials are electrical responses of the brain that are "evoked" in response to a stimulus. They are further classified as Stimulus Related Potentials and Event related Potentials.

Stimulus Related Potentials (SRPs) are records of the changes in electrical potentials in the nervous system in response to an external stimulus e.g. Auditory Brainstem Response, Visual Evoked Response etc. They are obligatory responses that are independent of attention or interest of the subject in the stimulus.

The term Event Related Potentials or ERPs refers to the responses evoked due to various mental workloads when a stimulus and the problem related with that stimulus are applied. They occur only when the subject is selectively attentive to the stimulus and are elicited in conditions where the subject has to distinguish a target stimulus from non target stimuli [16].

Components of ERPs

The long latency response to a rare auditory stimulus consists of different waves i.e. N1, P2, N2 and P3 (also called P300). The P300 component of ERPs was first described by Sutton in 1965 [17]. The N1 and P2 components are believed to reflect the activity in neural areas that are activated by sensory modality and are independent of the subject's attention [18]. The N2 component is related to the unexpectedness of the stimulus [19]. Thus any event related potential includes an early sensory evoked potential and a late (cognitive) response P300 component. Various other component waveforms have also been identified such as P165, N2 and P3a [19-21]. P300 (the positive wave occurring after 300msec of stimulus) is found more consistently than other waveforms. This occurs as the other components are of relatively small amplitude and thus more difficult to separate from background noise. Also these components occur at short latencies and overlap with the simultaneously occurring stimulus related potentials.

Various theories have such as Context Updating Theory, Wicken's Multiple resource model, Context Closure and Template matching have been put forward to interpret the meaning of P300 [22-25]. ERPs are a way of observing the functioning of the human brain as they allow the cognitive processes to be observed "from within" [26]. The most widely accepted views for interpretation of P300 are that it is evoked by unexpected stimuli, that it reflects the updating of working memory and that its amplitude indicates the amount of processing needed by a given stimulus.

Caffeine, CNS and Event Related Potentials

Various researchers have done studies to evaluate the effects of caffeine on central nervous system using EEG, psychological tests, reaction time, and of late evoked potentials. Hollingsworth in 1912 noted that 65-130 mg of caffeine improved typing speed. A dose of 390 mg resulted in poor motor performance and tremor [27].

Wolpaw and Penry examined the acute effects of 300 mg caffeine on N1P2 peak to peak amplitude in 31 subjects [28]. They found a decrease in N1P2 amplitude with placebo. This decrease was not seen with caffeine. Caffeine has been found to increase auditory vigilance in doses of 75-300 mg compared to a lactose dummy. In the same study it was seen that auditory reaction times were shortened, tapping rates increased and subjects felt more alert following caffeine consumption [29].

Tharion et al studied long latency evoked potentials during performance of visual vigilance task following

administration of 200 mg caffeine [30]. They found a significant decrease in P2-N2 amplitude after caffeine intake. Jarvis surveyed 9003 adults in UK and found that increased levels of coffee and tea were associated with improved performance on a range of cognitive tasks [31].

Study by Lorist et al demonstrated a decrease in RT and decrease in error rate following caffeine ingestion [32]. There was a more negative going N1 with a shorter latency. P3 amplitude revealed an increase with no significant change in its latency. Caffeine has been demonstrated to lead to a decrease in RT and increase in amplitude of visual evoked potentials. Azcona et al were of the view that this effect was not due to reversal of caffeine withdrawal as the subjects were not heavy caffeine consumers [33].

Lorist et al examined the effects of caffeine on specific information processing activities and on search processes [34]. They found that reactions were faster in focused attention condition than in divided attention condition. Caffeine led to a decrease in P3b (Stimulus evaluation processes) latency in focused attention and low display load conditions and thus accelerated stimulus evaluation. There was an increase in amplitude of N2b also. Kawamura et al investigated the effects of 500 mg caffeine on ERPs in 10 subjects [35]. The oddball paradigm showed that P300 amplitude and area were significantly increased after 30 minutes of caffeine intake. These effects disappeared after 210 minutes. P300 latency and Reaction time showed no significant change with oddball paradigm.

Another study by Lorist and Snel evaluated the effects of 3mg/Kg body weight of caffeine by using a visual selective attention task [36]. In the after caffeine condition the time taken to localize the target letter decreased and the information about the location of the target was passed earlier to the response system. The process of feature analysis was not affected by caffeine.

In their study Seidl et al found that Reaction time improved in response to target stimuli after administration of drink having caffeine [37]. The subjects showed shorter latencies of P300 though it was not statistically significant. A larger frontocenterally distributed P2 was found by Ruijter et al after caffeine intake [38]. The N2b component showed an increase in negativity. In the after caffeine intake condition the irrelevant target yielded a P3, thereby indicating that the subjects processesed the irrelevant target as well. There was a decrease in RT although the number of hits and false alarms showed no change.

Warburton et al evaluated the effects of caffeine on information processing using participants who had minimal deprivation from caffeine [39]. Their study also evaluated the effects of sugar using a sugar containing and a sugar free control drinks. A comparison of the two control drinks showed no significant difference, thereby indicating that glucose was not the substance responsible for improved performance in their study. The caffeine containing drink led to an increase in attention and verbal reasoning without affecting memory.

In a study on 30 subjects Yeomans et al observed a decrease in RT and increased response accuracy on a performance task [40]. These effects were however seen in subjects who were given caffeine after being in a caffeine deprived state. Preloading the subjects with caffeine had no significant effects on performance or mood. They suggested that the effects of caffeine were due to reversal of caffeine withdrawal condition.

Dixit et al evaluated the effect of caffeine on Event Related Potentials and reported non-significant decrease in latency of N1, P2, N2 and P3 after caffeine consumption [41]. The amplitude of P3 showed a significant increase after intake of caffeine. They concluded that caffeine led to facilitation of information processing and motor output response of the brain [41].

The consumption of caffeine has been shown to improve cognitive performance. There is increase in alertness and shorter reaction times. Thus caffeine leads to an overall improvement in cognitive functions. However more work is needed in the field to characterize if the improvement in cognition is global or in specific domains.

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