Original Article

EFFECT OF GRADE -III EXERCISE ON TOTAL LEUCOCYTE COUNT AND DIFFERENTIAL LEUCOCYTE COUNT

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ABSTRACT

Introduction: Exercise is a type of physical activity and is defined as a planned. Structured and repetitive bodily movement done to improve and to maintain physical fitness .exercise stress releases some amount of adrenaline from adrenal medulla. Adrenaline brings about a beta receptor mediated vasodilatation in skeletal muscles which may contribute to the anticipatory vasodilatation in the blood vessels, and increased blood flow is seen during and for a short while after the exercise. Due to the stimulation of bone marrow more number of white blood cells (WBC) produced from marginal pool and released into circulation.
Materials & Methods: Thirty subjects both male and female were randomly selected as control and study groups. Control group sample was collected after taking rest for 1 hour and study group sample was collected immediately after grade III exercise.
Conclusion: After exercise total leukocyte count (TLC) increased significantly p. value being < 0.0001 and differential leukocyte count(DLC) increased significantly p. value being < 0.01.
KEYWORDS: Marginal pool-Adrenaline-Grade III exercise-Random-Vasodilatation.

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INTRODUCTION

Work physiology is a branch of science which deals with the physiology of man at work. Muscular exercise is the highest expression of activities of the body and almost all resources of the body are mobilized in order to bring about the greatest efficiency of neuromuscular system. Muscular movement or exercise is a cortical phenomenon and is under the feed back control of cerebellum and cerebral cortex of the brain. Muscular exercise is also a form of physical stress. A man physically exerts to perform various tasks of life, for example, fight and flight reactions, feeding behavior, sports, climbing up and down the stairs, etc which may vary from mild to severe exercise and call for increased oxygen supply, removal of metabolic end products like co_2 and lactic acid and there by yielding appropriate energy to meet energy requirement of the active tissues. This is met by various bodily changes that occur in various systems. Blood cell changes like Neutrophilia[1,2], Eosinophilia[1,2], leucocytosis, lymphoctopenia, etc.

MATERIALS AND METHODS

The institutional ethical committee approval was obtained.

Investigative Procedure:

An investigative study was conducted at Mamata Medical College, Khammam. Thirty students who took admission for postgraduate course in Mamata Medical College, inclusive criteria are those who are willing to participate belonging to both sexes are randomly selected as subjects. Age group ranges between 20-35 years. Subjects without any health problems like haem-otological, Respiratory, Cardiovascular and other physical problems were excluded from the study. The procedure of exercise was explained to them and they were trained to perform the exercise a day before the experiment. The next day after breakfast they were asked to take rest for 1 hour. Under aseptic conditions 3ml of blood is drawn from accessible antecubital vein and transferred to a sterile vial containing 6 mg of double oxalate (anti coagulant)salt. The subjects were asked to mount the computerized treadmill inclined at 30^0 and the speed was set to 4km/hr. They were asked to walk on the treadmill. The pulse rate was raised to 125-150 beats/min and calories consumed to 6cal/min and the subject was asked to perform the exercise for another 5 minutes at the same rate. At the end of 5minutes treadmill was stopped and the subject was asked to dismount and immediately 3ml of blood was taken again in 6mg of double oxalate which is present in a sterile vial. The vial is gently rotated to mix the anticoagulant and blood well.

Total Leucocyte Count (TLC)

The method to determine count total number of leucocytes was by diluting the blood with Turk's fluid, charging the neubaeur's counting chamber and count the white blood cells under low power objective lens by using light microscope, take a Clean and dry the pipette, watch glass, cover slip and neubaeur's counting chamber thoroughly. Take enough Turks fluid in a watch glass. Suck the oxalated blood exactly up to the 0.5 mark of WBC pipette. And immediately Suck Turk's fluid exactly up to the mark 11of WBC pipette Hold the pipette horizontally and close in both ends , then gently mix rotate the contents in the bulb of pipette. Discard the first few drops of fluid from the pipette as the fluid in the stem does not contain cells .Charge the neubaeur's counting chamber and allow to ten minutes for the cells to be settle down in neubaeur counting chamber then Focused on low power objective lense of the light microscope and count the total number of WBC's in all four corner squares of neubaur counting chamber

Differential Leucocyte Count (DLC):

For the counting of WBC used Leishman's stained method Making a blood smear is Take four clean and grease-free glass slides and select one as spreader .Place a drop of oxalated blood on one end of a slide in the middle about 1cm from the end, by touching the slide to the top of the blood drop.With the help of a smooth clean edge of the spreader making an angle of 30^0-45^0 touch the blood drop, then the blood drop spreads across the length of the spreader, push the spreader to the other end of the slide with a smooth, quick, and controlled movement. A thin layered smear will be formed.2-3 smears of such were prepared. Dry the blood smear quickly by waving the film in the air.

STAINING PROCEDURE is Place the slides on the staining rack with the blood smear facing up. Pour 8-12 drops of Leishman's stain on the slide, The stain should just cover the smear, Leave it for 2 minutes. During this period, the alcohol in the stain fixes the cells (fixation time) after 2 minutes add double the amount of distilled water on the smear, with the help of a dropper without spilling until a greenish scum or metallic shiny layer is formed. Mix the stain and the water evenly by blowing gently or by shaking the slide .Leave it for 8-10 minutes-(staining time). After 10 minutes overflow the stain with distilled water, and wash the slide gently and thoroughly under tap water. Shake off all water adhering to the slide and set the slide in an inclined position to dry .Focus under low power first and turn to oil immersion, count and identify the cells.

RESULTS AND TABLES

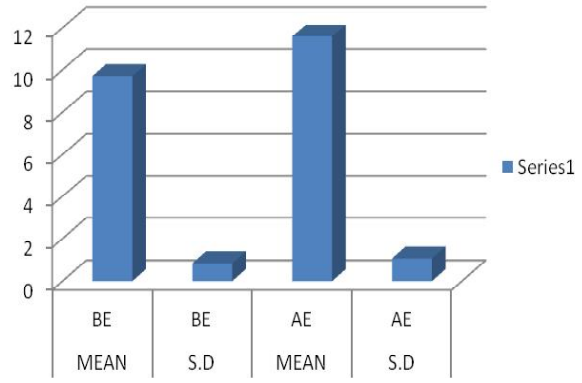
Statistical analysis;

The data of total leucocyte count and defferiential leucocyte count table-1and table-2 Statistical analysis was done by using Microsoft Excel and values were expressed as Mean and Standard Deviation (SD) of each parameter Statistical significance level was evaluated by probability test (P values<0.01).

Table 1: Total leucocyte count (TLC).

MEAN	S.D	MEAN	S.D	P. value
BE	BE	AE	AE	<0.0001
9.71	0.84	11.58	1.07	

Graphical representation of Table 1.

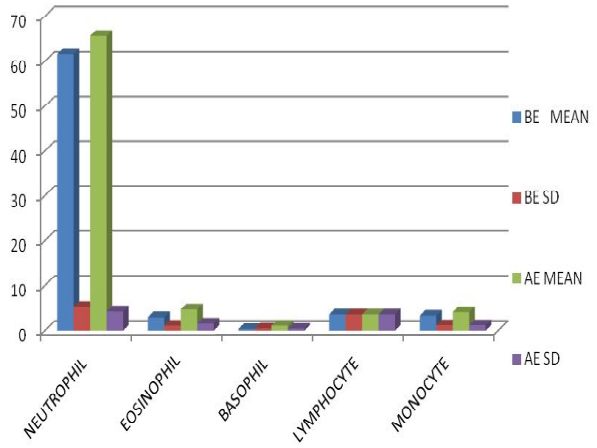


BE: Before Exercise AE: After Exercise

Table 2: Defferential leucocyte count (DLC).

PARAMETERS	BE MEAN	BE SD	AE MEAN	AE SD	P-VALUE
NEUTROPHIL	61.63	5.18	65.63	4.21	<0.01
EOSINOPHIL	2.97	1.1	4.67	1.58	<0.01
BASOPHIL	0.4	0.5	1.1	0.48	<0.01
LYMPHOCYTE	3.58	3.58	3.58	3.58	<0.01
MONOCYTE	3.3	1.21	4.07	1.23	<0.01

Graphical representation of Table 2.



BE: Before Exercise AE: After Exercise

DISCUSSION

Total Leucocyte Count (TLC) increases during exercise, **table -1** shows It is highly significant p valve (p<0.0001) as in the physical exercise, circulation becomes more dynamic. The leucocytosis [2,3,4,5] caused by exercise in this study could be due to a number of factors like, demargination, the actions of catchecholamines, cortisol, neuronal transmitters and peptides or purine chemical transmitters (Mc Carthy and Dale-1988)[6,7]. A possible explanation for the post-exercise increase in leucocyte count is that large number of cells which at rest, are adherent to the walls of blood vessels (endothelium),i.e., marginated pool are suddenly washed into circulation (Morehouse and Miller, 1976)[8] which is called demargination and results in massive increase in the number of leucocytes in the circulating pool. The demargination is due to the force of increased volume and velocity of blood flow (Morehouse and Miller, 1976)[8]. Demargination could be also due to the effects of hormones, such as adrenaline[9], which decreases the adherence of leucocytes to the endothelium via interaction withδ receptors on both cell types (Gleeson, 1995)[10]. As a result TLC increases; there occurs Neutrophilia[1], Eosinophilia[2], Basophilia, Monocytosis and relative Lymphocytopenia. [11]

Differential Leucocyte Count (DLC) of Neutrophil Count and it is increased during exercise, table-2 shows It is significant of p value (p<0.01). The increase in neutrophils may be due to release of the hormone adrenaline[9] with the immediate mobilization of neutrophils that have marginated in small blood vessels or lungs, spleen, lymphatic tissue and hematopoietic bone marrow, which enter the circulation and increase the cell count mobilization of neutrophils from the margin-ated pools into the peripheral circulation is mediated by the exercise intensity-dependent secretion of stress hormones, such as catecholamine's, cortisol and growth hormone. Catecholamine's wash out neutrophils from the marginated pools into the circulation through sheer force due to exercise-induced hemodynamic. Cortisol mobilizes neutrophils from the bone marrow reserves(Mutsuo, Yamada, Katushiko)[12]. The cytokines Granulocyte Colony Stimulating Factors (G-CSF) [12] and Granulocyte-Macrophage Colony Stimulating Factors (GM-CSF) stimulate marrow production of neutrophils and can cause dramatic elevation in the WBC's. Suzuki et al (1995)[5] also reported about Neutrophilia of greater magnitude after severe exercise.

Eosinophil Count: Eosinophil count increases during exercise. It is significant of p- value ($p<0.01$). Increase in eosinophils may be due to release of histamine from basophils[13]. Histamine may act as chemo attractant which may cause migration of eosinophils from various sources as they got concentrated around histamine and histamine concentration is more in blood. Eosinophilia after exercise was observed by Moyana et al (1996)[14] and Hanson and Flaherty (1981).[15]

Basophil Count: Basophil count also increases during exercise. It is significant of p-value is ($p<0.01$). Increase in basophils may be due to demargination and release from bone marrow by various stimuli like corticoids, adrenaline etc. during exercise histamine is released into circulation. The cells most implicated in blood histamine release are basophils.[13]

Lymphocyte Count: Lymphocyte count decreases during exercise. It is significant of p- value ($p<0.01$). The decrease in lymphocyte count may be a relative decrease as others neutrophils, eosinophils, basophils and monocytes increase is significant. Strenuous exercise is associated with an increase of serum cortisol[12] resulting in lymphocytopenia[11]. It may also be due to stress and released corticosteroids however the stress is of very short duration to cause lymphocytpenia Suzuki et al (1995)[5] also observed peripheral lymphocytopenia after termination of exercise and the changes persisted for one hour, even after termination of exercise. Gleeson et al (1987)[10] also reported that proportion of lymphocytes to granulocytes decreased after exercise.

Monocyte Count: Monocyte count increases during exercise. It is significant of p-value ($p<0.01$).The increase in monocytes may be due to demargination, the effect is mediated by beta2 adrenergic receptors and probably due to change of the avidity state of adhesion molecules (Holger HW. Gabriel and Wilfred kindermann-1977)[16] The increase in monocytes may also be due to release of cytokines, GranulocytesMacrophage colony stimulating factors released as a result of relative hypoxia that the bone marrow production of monocyte increases and cause elevation of monocytes.

An increase in monocytes, immediately after exercise is also reported by Gabriel and Kindermann (1977)[16], Moyana et al (1996)[17] and Boas et al (1996) reported an initial increase and then a decrease to baseline, 1-2 hours after exercise. The graphical representation of DLC all parameters are showed by table –2 graph.

CONCLUSION

It can be concluded from this study that during vigorous exercise of short duration there is a significant rise of total WBC count[18], Differential Leucocyte Count[9] of Neutrophils, Eosinophils, Basophils and Monocytes. The various cells may be released into circulation either from red bone marrow or by demargination[18] of cells from marginated and tissue pools and by deseqes-tration of cells from blood stores like spleen, lungs and splanchnic blood vessels, under the influence of multiple factors that may operate during exercise like interleukins IL-6[5], IL-3 , and IL-5,GM-CSF[5], Gluco-corticoids,Adrenaline[19] and histamine. The purpose appears to be:

· To meet the raised o_2 demand by raised metabolic rate and the need to wash out metabolites.

· To increase first and second line of defence and raise body resistance which may fall during vigorous exercise.

There is also significant decrease in lymphocytes in circulation and which may either be a relative one as other cells rise or due to sequestration of these leucocytes in sluggish lymphatic circulation since the exercise is of shorter duration. The stress phenomenon may not be the cause of decrease in lymphocytes and which is supported by the fact that there is increase in eosinophils in circulation during exercise.

Conflicts of interest: None

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