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RESEARCH ARTICLE

Influence of Aminotransferase Enzyme on Delayed Onset Muscle Soreness among Novice Athletes: A Pilot Single-Blind Randomized Clinical Trial

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ABSTRACT:

Introduction: Delayed onset muscle soreness (DOMS) is a familiar experience for the elite as well as the novice athlete. One of the most popular concepts in the lay exercise community is that delayed soreness is a result of metabolic waste product accumulation in the muscles. **Objective:** To evaluate the pattern of changes in serum aminotransferase level after performing a different intensity of eccentric exercises and to find a suitable percentage of one repetition maximum (1RM) to be selected for inducing delayed onset muscle soreness (DOMS). **Materials and Methods:** A total of 48 novice athletes was selected and a simple random sampling technique with the lottery method was adopted to participate in this three group, a pilot single-blind, randomized clinical study. After collection of demographic data, the athletes were allotted to the group based on the chits what they have picked. Group A consists 70 % of 1 RM, Group B consist 80 % of 1 RM and Group C consist 90 % of 1 RM taken for the induction of Delayed Onset Muscle Soreness (DOMS) experimentally. Blood samples were collected at the baseline and 24 hours,48 hours,72 hours & 96 hours after inducing DOMS. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were considered as dependent variables and values displayed in the instrument were noted. **Results:** Repeated measures ANOVA and one-way analysis of variance were adopted to analyse the statistical difference between the groups. The p-value less than 5% (0.05) is considered to be significant. There exists no significant difference between the three groups at baseline to 24 hour measurements at ($P > 0.05$). On the other hand, a statistically significant difference was found between three groups at 48 hours,72 hours & 96 hours at ($P < 0.05$). **Conclusion:** Based on the changes occurred in serum aminotransferase levels, the study concluded that 80% of 1 RM were experimentally a standardized procedure for the induction of DOMS.

KEYWORDS: Delayed Onset Muscle Soreness, Repetition Maximum, Alanine aminotransferase, Aspartate aminotransferase.

INTRODUCTION:

Delayed Onset Muscle Soreness (DOMS) described as a most common muscle pain experienced by a person after performing a strenuous exercise that involves a lengthening contraction of muscle. The soreness will begin to occur around 8 to 24 hours after eccentric exercise and will peak at 48 to 72 hours post exercise [1]. The muscle soreness is mostly experienced upon a

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muscle action or through the palpation of the muscle. At rest, there is a minimal perception of muscle soreness and generally decreased at 72 to 96 hours with mild soreness remaining than the specified time frame [2].

Many athletes have experienced DOMS, if a novice athlete have not trained for six to eight weeks, or who modify their training regime to incorporate new method and individuals who initiate a training program are also at risk for developing DOMS [3]. Negative implications of muscle soreness includes mild to severe soreness, unable to continue protective and effective training, abnormal mechanics predisposing to injury, decreases in muscle strength and power, and a decreased confidence, motivation and willingness to undergo training session due to the negative effects [4].

Evidence suggests that a bout of lengthening contraction of a muscle will result in some form of adaptation in the exercised muscle. It has a beneficial and protective effect in other bouts of lengthening contraction of the same muscle and microscopic structural muscle injuries are significantly minimized [5]. It is evidenced that during the repair and recovery process both muscle and connective tissue are strengthened and; thus, more resistant to further microscopic muscle injury [6]. There are various theories describing the reason for the delayed onset muscle soreness. In, an attempt to minimize DOMS, it is important to understand the underlying mechanisms contributing to DOMS. These mechanisms include muscle contractile element damage and association of its connective tissue, as well as process of inflammation associated with micro trauma to the contractile and non contractile properties [7].

An excess metabolite theory proposed that after performing an exercise, there is an accumulation of lactic acid, which is the reason for the muscle soreness, but the theory was rejected due to the maximum degree of metabolism occurred in homocentric contraction [8]. Hough (1902) initially projected the muscle injury theory that the rupture of muscle fibre takes place as a result of actin myosin changes brought about by concerned lengthening contraction of muscle which leads to the development of tissue damage. The micro injury stimulates flow of leukocytes to the area in response to inflammation and leads to the discharge of prostaglandins and histamines that activates the pain receptors. This stimulates the nociceptors, which increases the serum activities of the enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH) as a result of sarcomere disruption involving maximum membrane permeability [9].

In contrast, skeletal muscle contains isoenzymes of CK and LDH which may be released into the blood stream following a muscle injury. Although, reports of elevations in CK-MM found primarily in skeletal muscle and LDH levels in subjects with skeletal muscle damage exist in the literature and increase in these levels along with elevations of intracellular molecules indicate damage to the sarcolemma following eccentric exercise [10]. After strenuous exercises, skeletal muscle enzyme changes have been reported in normal subjects and athletes. In these instances, a high serum enzyme activity is a consequence of sarcolemmal membrane damage and is related to the severity of muscle soreness [11].

On the other hand, there are liver enzymes such as Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) which is present in all human tissues and large amount of enzymes were found in the liver, heart, kidney and skeletal muscle [12]. The levels of aminotransferase enzymes are increased in case of inflammatory myopathies, but elevations occur less frequently than creatine kinase level [13]. In fact, skeletal muscle has more aminotransferase enzyme when compared with that in the liver because of a larger tissue mass [14]. There is a paucity of research to explore an association between skeletal muscle damage and sequence of variations in liver isoenzymes following sternuous exercises [15]. Therefore, the study evaluated the pattern of changes in serum aminotransferase after performing a different intensity of eccentric exercises among novice athletes and also found a suitable percentage of one repetition maximum (1RM) to be selected for inducing delayed onset muscle soreness (DOMS).

MATERIALS AND METHODS:

Recruitment and Allocation:

The university researchers and institutional ethics committee have approved the research protocol (2017/0151/PT17D006) and Helsinki declaration, revised 2013 guidelines was strictly followed in the study [16]. A power analysis using nMaster 2.0 software for repeated measures indicated that an alpha of .05, a power of 80%, an effect size of 0.8, and a sample size of 48 were needed. A total of 48 novice athletes was selected and simple random sampling technique with the lottery method was adopted to participate in this pilot, randomized clinical trial with single blind, multi group repeated measures design [17]. The study was conducted from may 2018 to July 2018 at Dr. M.G.R. Educational and Research Institute, Chennai. The study sample size was 16 per group to be sufficiently powered to conduct a pilot study [18]. Male novice athletes [19] with age group between 18-25 years, who were not under any specific training protocol and no recent history of upper extremity musculoskeletal injury were taken as inclusion criteria for the study and athletes, who had elevated

aminotransferase parameters at baseline measurements were excluded from the study [20]. After collection of demographic data, the athletes were allotted to the group based on the chits what they have picked. Group A consist 70 % of 1 RM, Group B consist 80 % of 1 RM and Group C consist 90 % of 1 RM taken for the induction of DOMS experimentally.

After the calculation of one repetition maximum, 70%, 80 % & 90 % of 1 RM was calculated using epley formula [21] for each subject based on the allocation for inducing DOMS. Shortening contractions of elbow flexors were followed by eccentric contractions for five seconds. During concentric contraction of elbow flexors, assistance was given by an investigator and no assistance for eccentric contractions of biceps brachii muscle (elbow extension) and verbal encouragement was given. All subjects should perform four sets, one set consisting of ten repetitions with a rest period of 3-5 minutes between each set [3].

Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST):

The procedure is based on principles outlined by (wroblewski & ladue) and utilizes a modification of the methodology recommended by the International Federation of Clinical Chemistry (IFCC) [22]. Erba Chem 5 plus instrument was used for the analysis. Kinetic and electrochemical method of analysis of blood samples based on catalyzed and enzyme reactions provides an internal consistency between the measures [23]. The kinetic reaction type was adopted for all the subjects. Then 0.05 ml of serum added to the 1 ml of AST reagent. Similarly, 0.05 ml of serum added to the 1 ml of ALT reagent, and incubated at 37 degree Celsius for 60 seconds in an incubator [12]. The normal concentrations in the blood for males are up to 40 units per litre (U/L) for the AST and up to 55 units per litre (U/L) for ALT [24]. Blood samples were collected at the baseline and 24 hours, 48 hours, 72 hours & 96 hours after inducing DOMS and values displayed in the instrument were noted. In this study, all novice athletes baseline parameters were within the reference range and none of them were dropped out.

The collected demographic data were analysed using IBM (Armonk, NY: IBM Corp.) of statistical package for social science SPSS version 20.0. Shapiro-Wilk test was used for testing normality of data as the study sample size is below 50 ($n < 50$). The demographic features of novice athletes were displayed in [Table 1]. The descriptive data were analysed and their distributions are expressed in terms of mean ± standard deviation. Repeated measures ANOVA was adopted to analyse the statistical differences within the groups and one way analysis of variance was used to find out the difference between the groups. p-value less than 5% (0.05) was considered to be significant [25]

Table 1:Demographic characteristics of the subjects recruited in Group A, Group B & Group C

Parameters	Group A	Group B	Group C	p value
N	16	16	16	-
Age	21.5 ± 2.8	20.7 ± 3.1	21.4 ± 2.9	0.80
Height (cm)	160 ± 3.6	162 ± 5.9	164 ± 4.1	0.67
Weight (kg)	64.4 ± 6.0	66.7 ± 7.1	67.2 ± 3.1	0.50
BMI (Kg/m^2)	22.3 ± 2.7	21.9 ± 3.5	23.4 ± 1.6	0.89

Abbreviations: cm-centimetre; kg-kilogram; BMI-Body Mass Index

RESULTS:

Forty eight novice athletes were recruited for the study. One way analysis of variance test was used to compare the mean values between Group A, Group B & Group C.

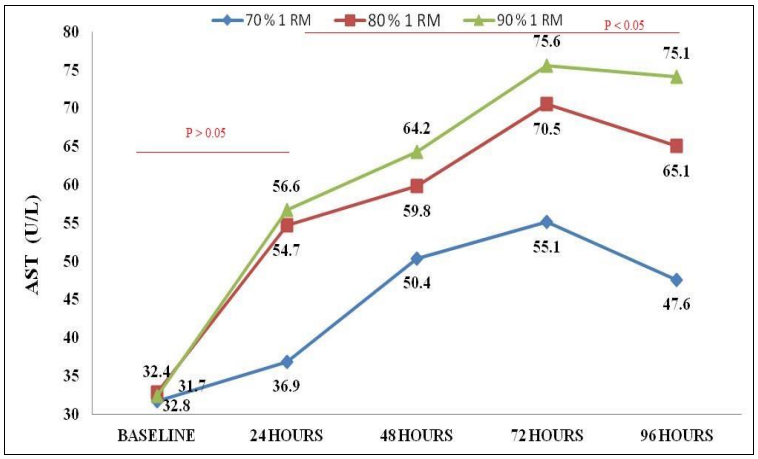

Figure 1: Aspartate aminotransferase (AST) mean values comparison at baseline, 24 ,48,72, & 96 hours using one way analysis of variance

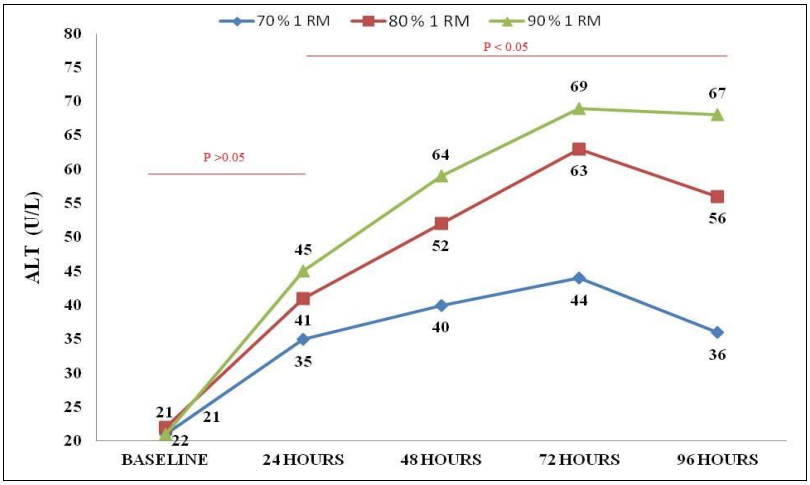

Figure 2:Alanine aminotransferase (ALT) mean values comparison at baseline, 24, 48,72, & 96 hours using one way analysis of variance

Table 2:Summary of AST repeated measures ANOVA findings

Dependent VariableAST (U/L)	70% 1RM		80% 1 RM		90% 1 RM	
	MEAN	S.D	MEAN	S.D	MEAN	S.D
Baseline	32.8	5.66	31.7	5.09	32.4	4.37
24 Hours	36.9	4.91	54.7	2.86	56.6	2.15
48 Hours	50.4	2.71	59.8	2.91	64.2	2.67
72 Hours	55.1	2.46	70.5	2.63	75.6	1.12
96 Hours	47.6	1.76	65.1	2.52	75.1	1.36
Anova f Value	184.72		357.10		803.31	
p value	0.00**		0.00**		0.00**	

Note: * p >0.05(Not Significant), **p < 0.05(significant), U/L-Unit Per Litre

Table 3:Summary of ALT repeated measures ANOVA findings

Dependent Variable ALT (U/L)	70% 1RM		80% 1 RM		90% 1 RM	
	MEAN	S.D	MEAN	S.D	MEAN	S.D
Baseline	21	5.46	22	5.15	21	5.45
24 Hours	35	4.96	41	2.76	45	2.16
48 Hours	40	2.11	52	2.19	64	2.43
72 Hours	44	2.06	63	2.45	69	1.14
96 Hours	36	1.85	56	2.59	67	1.27
Anova f Value	109.61		211.40		531.90	
p value	0.00**		0.00**		0.00**	

Note: * p >0.05(Not Significant), **p < 0.05(significant), U/L-Unit Per Litre

For both, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels, there exists no significant difference between the three groups at the baseline to 24 hour measurements at (P > 0.05) and, statistically significant difference was found between three groups at 48 hours,72 hours & 96 hours at (P < 0.05). [Figure 1] & [Figure 2]. Anova with repeated measures showed that the AST and ALT measures overall changes within three groups at (P < 0.05). [Table 2] & [Table 3].

DISCUSSION:

The intent of this study was to find the effects between three different DOMS inducing protocol for elbow flexors by eccentric loading exercise in association with a sequence of changes in serum alanine aminotransferase and aspartate aminotransferase levels. An elevation in liver enzymes following strenuous physical activity has been reported previously. In this study, three different DOMS inducing protocols were compared to facilitate the normal physiological process after strenuous exercise and time is taken for the recovery process. Generally, DOMS appears initially from 8 to 24 hours after eccentric exercise and peaks at 48 to 72 hours post exercise. The present study has investigated the various exercise intensity for elbow flexors and its physiological response in the human body. The primary objective of the study was to select an appropriate percentage of 1 RM for the standardization of DOMS inducing protocol.

In this study, the baseline parameters of liver enzymes such as serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were within the reference range of the subjects in all three groups. However, ALT & AST liver enzymes were elevated in

all subjects after the induction of DOMS. Thapa et al (2007), evidenced a classification in the levels of aminotransferase enzymes in response to strenuous exercises with other liver disease [26]. The results evidenced that there was no significant difference in the dependent variables between baseline parameters and 24 hour measurements at ($P > 0.05$). Numerous studies have reported that circulating white blood count elevates within 8 hours after a lengthening contraction exercise, but mostly, it depends on the exercise intensity, duration and type of muscle contraction [27]. In this study, we found that there was a significant difference in the ALT & AST between 48 hours to 96 hours of time course with greater elevation in the liver enzymes at 72 hours in all three groups at $P < 0.05$. An eccentric exercise with a varying intensity produces a sequence of physiological changes in an involved muscle. To understand the physiological theories associated with DOMS, certain ideas have been evidenced. These changes are due to muscle tissue tear with a discharge of some chemical enzymes and inflict the development of osmotic pressure, which results in pain, swelling, muscle spasm and decreased muscle performance[8]. Fallon et al (1999), concluded that a significant elevation in the levels of liver enzyme was based on the duration and intensity of training along recovery time of muscle following eccentric exercise [28].

Levinger et al (2009), concluded that the gold standard protocol for assessing muscle strength was through one repetition maximum (1RM) in non laboratory situations. Numerous studies have evidenced that the 1RM testing is safe for the participants to assess muscle strength [29]. Based on the dependent variable results, it was found that 70%, 80% & 90% 1RM have detected markedly elevated serum alanine aminotransferase and aspartate aminotransferase between 48 - 72 hours following DOMS. During submaximal exercises, repeated contraction of muscle uses energy substrates and tissue metabolism was within membrane permeability. However, when the intensity of exercise exceeds the membrane permeability, it leads to sarcolemma structural alteration resulting in creatine kinase release with buildup of amine and kinins that activates the cellular enzyme along nociceptors and leading to a feeling of muscle soreness [30].

Hence, therefore the enzymatic level of alanine aminotransferase and aspartate aminotransferase were markedly elevated after 48 hours in Group B (80% 1RM) and Group C (90% 1 RM) after inducing DOMS. In case of Group A (70% 1 RM) both ALT & AST enzymatic levels were within the reference range till 48 hours and drastically declined between 72 - 96 hours. The greatest elevation was observed in Group C (90% 1 RM) after 48 hours following eccentric exercise and the enzymatic values remain continued even until 96 hours. In case of Group B (80% 1RM) the ALT & AST levels were elevated between 48 - 72 hours, then the values started to decline towards the reference range ALT (56 U/L) and AST (65.1 U/L). Interestingly, this study has found that 80% 1 RM was a suitable method and a standard procedure for the induction of DOMS.

CONCLUSION:

The pilot study concluded that 80% of 1 RM were experimentally a standardized procedure for the induction of DOMS using eccentric loading protocol and also it limits the levels of alanine aminotransferase and aspartate aminotransferase enzymes at 48 to 72 hours when compared to 70% and 90% of one repetitive maximum.

LIMITATION AND FUTURE RECOMMENDATIONS:

Only male novice athletes were participated. Serum aminotransferase levels were considered as a dependent variable. Delayed onset muscle soreness was induced to the elbow flexor muscle group alone. It is recommended that the future research studies can be done to observe the changes over calcium channel blockers, muscle strength, pain, swelling, and tenderness. Based on the pilot study report, a larger subsequent study can be undertaken.

DECLARATION OF INFORMED CONSENT:

The authors certify that they have obtained all appropriate consent forms from the subject regarding his blood sample values and other clinical information to be reported in the journal.

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AUTHOR'S CONTRIBUTIONS:

Selvaraj Sudhakar - conducted the research work, collected & organized data. Jibi Paul- designed the research study and interpreted the data. Senthil Selvam P - provided the logistic support for the study. Mahendranath P - helped with data collection and literature search. All authors have drafted the article and finalized the manuscript.

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