Original Article

Can We Use Creatine Kinase Muscle Type as a Potential Marker for Muscle Viability in Mangled Extremities? A Preliminary Evaluation of its Applicability and a Literature Review

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Abstract

Objectives: Increased expression of serum creatine kinase muscle type (CK-MM) in muscle overuse or injury has been documented in many situations. However, the expression of CK-MM and its correlation with the extent of traumatic damage to skeletal muscle tissue has not been explored. We studied the pattern of expression of CK-MM in substantially damaged muscle tissue and attempted to correlate its expression with the extent of muscle damage. **Methods:** At level 1 trauma center, 15 patients with mangled lower limb were prospectively evaluated. All patients underwent primary amputation (mangled extremity severity score ≥7) using specific criteria. Muscle tissue samples were obtained intraoperatively from 3 different zones (chosen arbitrarily based on clinical parameters of muscle viability, Zones A, B, and C) for all patients. Samples were evaluated by hematoxylin and eosin (H and E) staining and immunohistochemistry (IHC) staining with polyclonal CK-MM antibody. **Results:** H and E staining correlated with the clinical extent of muscle death in Zones A, B, and C; the percentage of viable muscle fibers was 6.7% in Zone A, 20% in Zone B, and 73% in Zone C. Least CK-MM expression was noted in muscle tissues on IHC in Zone A (most necrotic area) and most in Zone C (most viable area). The score developed by us corroborated with the extent of muscle damage and viability. **Conclusion:** IHC staining for CK-MM can be used as a definite biomarker of muscle integrity and can be used as an adjunct to clinical evaluation to help define limb viability as well as levels of amputation when that is required.

Keywords: Amputation, biomarker, CK-MM, hematoxylin and eosin staining, immunohistochemistry, skeletal muscle tissue

Introduction

In an attempt to improve the understanding of tissue trauma, inflammation, and repair processes, the role of biomarkers has been evaluated over the past few decades.[1-12] Concerning muscle tissue trauma, most decisions about muscle viability are based on clinicoradiological parameters, predictive scoring systems, and the surgeon's clinical experience. However, in the new millennium, the widening of horizons at the molecular and genetic level has given us the ability to use biomarkers as indirect evidence to predict tissue viability.

Creatine kinase (CK) is one such biomarker that forms an integral part of skeletal muscle; it is a dimeric enzyme, found in both cytosol and mitochondria of muscle fiber. It forms a core functional unit for all chemical reactions and chains occurring in muscle fiber and helps to maintain its structural and functional integrity.[1] Cytosolic form of CK is composed of two subunits, designated as M (muscle type) and B (brain type). These subunits combine to produce three isoenzymes: MM (skeletal muscle), MB (cardiac muscle), and BB (brain tissue); the distribution pattern of these isoenzymes depends on the type of tissue.[2] The skeletal muscle is typically comprised of around 98% CK-MM and 2% CK-MB. In addition, CK-MM accounts for a significant percentage of total and serum CK activity, which is normally found in the serum of healthy adults.[3] The serum level of skeletal muscle enzymes

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is a potential marker for the functional status of muscle tissue and varies widely in both pathological and physiological conditions. CK activity in the blood is, therefore, believed to be the reflection of muscle membrane integrity.[4]

Various studies have correlated and established the increased expression of CK-MM activity in serum; this is in response to variable insult and injury to the skeletal muscle, more so in athletes.[5-17] The existing literature has already defined CK-MM isoform as a marker of myopathies or exercise-induced muscle damage.[5,6] Even in crush syndrome or prolonged exposure to cold, serum CK activity is markedly increased and can thus be used as a prognostic tool.[7-9] Some authors had even studied the variable expression of tissue CK activity in marathon runners by skeletal muscle biopsy.[18]

The use of tissue CK activity as a biomarker to predict the extent of muscle damage after the injury has not been mentioned in the published literature in English. We feel that there is some potential in evaluating tissue CK-MM activity after the injury to skeletal muscle tissue, more so in mangled limbs, where damage to muscles is significant, and there is a potential to use this as a prognostic viability tool.

Keeping that in mind, we designed this study in an attempt to evaluate the extent of tissue damage in an indirect way and to see if it is possible to predict tissue healing and survival.

Materials and Methods

This prospective cohort study included 15 patients (aged 18 years or more) presenting with a mangled extremity to the Advanced Trauma Centre of the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, over 1 year. Starting January 2016, patients of age ≥18 years, with both isolated injury and polytrauma, involving either upper or lower extremity and who were planned for amputation based on mangled extremity severity scoring system (≥7), were included for evaluation. Patients suffering from any known autoimmune disorder, immunodeficiency disorder, peripheral vascular disease, vasculitis, diabetes, morbid obesity, or malnourishment were excluded. Approval from the institutional ethical review board was obtained, and informed consent was taken from all participants.

Three different zones in the mangled limb were identified clinically; this was based on the color, consistency, contractility, and morphology of involved muscles. Zone A comprised of muscles from the actual mangled part of the limb, Zone B was the intermediate zone between Zones A and C with the tissue of doubtful viability, whereas Zone C consisted of proximal healthy and viable muscle through which the final resection of the limb was done. Skeletal muscle samples were taken from each zone separately [Figure 1]. All the biopsy samples from these patients were stored in 10% buffered formalin and processed further. Hematoxylin and eosin (H and E)-stained sections were used for the baseline study of the cases; interpretations with respect to tissue viability, integrity,

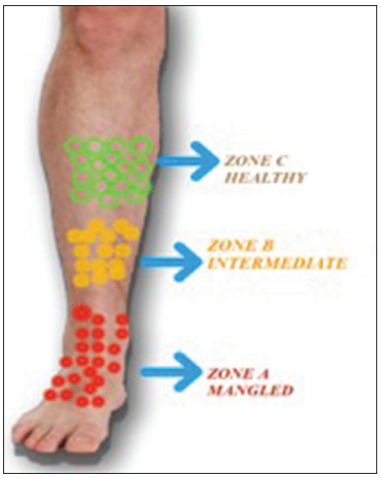

Figure 1: Pictorial representation of different zones chosen intraoperatively. The boundary between various zones was taken arbitrarily, based on clinical discrimination of healthy and injured tissue

and inflammation were done; and the special comment was made on the extent of degenerated muscle fibers with altered morphology and intercellular edema with interspersed inflammatory cells. Immunohistochemistry (IHC) was carried out by peroxidase antiperoxidase method using two micron-thick paraffin sections mounted on poly-l-lysine-coated slides. Rabbit polyclonal antibody in a dilution of 1:200 was used, after standardization of antibody.

The CK-MM antibody stains only intact and viable muscle fiber. Therefore, staining of CK-MM was expected to stain viable tissue only. The scoring of CK-MM was designed to evaluate the loss of stain intensity and pattern of involvement of muscle fibers; samples with less viable tissue were expected to have higher combined CK-MM scores and more loss of staining.

Loss of the staining was interpreted in context to normal control muscle, and the degree of staining was added to the evaluation, leading to a special 2-part scoring system as follows:

a. The pattern of muscle fiber involvement was scored into three types subjectively:
    1. Only scattered individual fibers showed a loss of staining
    2. A larger group of muscle fibers showed a loss of staining
    3. Very large group or whole muscle fascicle showed loss of staining.
b. The intensity of staining retained by the affected muscle fibers was scored into three types subjectively:
    1. A mild degree of loss of staining
    2. A moderate degree of loss of staining
    3. Total loss of staining.

The two scores were added up for the combined score of CK-MM for each biopsy. Thus, the minimum combined score of 2 meant that only scattered individual muscle fiber showed a mild degree of loss of staining (good viability, good prognosis). The highest possible combined score of 6 was interpreted as

a large group or the whole fascicle, showing a total loss of staining (poor viability, poor prognosis).

The data obtained were analyzed using appropriate statistical tests. All discrete categorical data were represented in the form of either a number or percentage, whereas the continuous data, which were normally distributed, were written in the form of its mean and standard deviation. If the data were skewed (ordered categorical data), it was represented in the form of its median and interquartile range. The normality of quantitative data was checked using measures of Kolmogorov–Smirnov tests of normality. The Mann–Whitney test was employed when two groups of skewed data were under consideration. In the case of normally distributed data, the means of two groups were compared using independent *t*-test. Proportions were compared using either the Chi-square test or Fisher's exact test, depending on their applicability. McNemar test was applied for comparison between categorical values of different zones. Whereas, the Wilcoxon signed-rank test was used for categorical data. All the statistical tests were two-sided and were performed at a significance level of $\alpha = 0.05$. The statistical analysis was performed using IBM SPSS Statistics (version 22.0, IBM corportaion, India).

An extensive literature search was done on PubMed [Table 1] using the following search string ("creatine kinase"[MeSH Terms] OR ("creatine"[All Fields] AND "kinase"[All Fields]) OR "creatine kinase"[All Fields]) AND (mangled [All Fields] AND ("extremities"[MeSH Terms] OR "extremities"[All Fields] OR "extremity"[All Fields]) but no relevant literature could be traced. Further literature search with different MeSH term ("creatine kinase"[MeSH Terms] OR ("creatine"[All Fields] AND "kinase"[All Fields]) OR "creatine kinase"[All Fields]) AND (("crush injuries"[MeSH Terms] OR ("crush"[All Fields] AND "injuries"[All Fields]) OR "crush injuries"[All Fields] OR ("crush"[All Fields] AND "injury"[All Fields]) OR "crush injury"[All Fields]) AND ("extremities"[MeSH Terms] OR "extremities"[All Fields] OR "limb"[All Fields]), 22 articles were identified. After detailed screening of abstracts and bibliography, no relevant study regarding CK-MM expression in skeletal muscle in crushed or mangled limb could be found.

Table 1: Search methodology

PubMed (1950-4/2019)	Number of Hits
CK	36,499
CK and muscle injury	3280
CK and skeletal muscle injury	842
CK and immunohistochemistry stain	73
CK and crushed limb	22
CK and mangled injury	0

CK: Creatine kinase

Results

The average age of patients was 40 years (range 20–70 years), and 2 of the 15 patients were females. All patients were involved in major accidents, with road traffic accidents being the most common mode of injury [Table 2].

On examination of H and E-stained tissue sections with CK-MM antibody, the following microscopic findings were noted.

Hematoxylin and eosin-stained sections

Sections from Zone A showed diffuse necrosis in 100% samples. In Zone B, 86.6% of sections had necrotic muscle fibers, which was further reduced to 40% in Zone C. This decrease from Zone A to C and from Zone B to C was statistically significant, with $P = 0.004$ and 0.016, respectively. Likewise, 6.7% of sections in Zone A had traces of viable muscle fibers which increased to 20% in Zone B and 73% in Zone C. This increase from Zone A to C and Zone B to C

Table 2: Epidemiological data of patients

Patients	Age	Sex	MOI	DIAGNOSIS	MESS
1	27	M	Railway tract injury	Lt traumatic knee disarticulation & Rt B/K mangled limb	10
2	40	F	RTA	Lt mangled foot with DNVD	11
3	45	F	RTA	Rt B/K mangled limb with Lt Crush Foot	11
4	65	M	RTA	Rt mangled upper limb with DNVD	11
5	60	M	RTA	Lt B/K mangled limb with DNVD	13
6	35	M	RTA	Rt B/E mangled limb with DNVD	9
7	55	M	Railway tract injury	Lt A/k mangled limb with Rt B/K traumatic amputation with DNVD	13
8	36	M	RTA	Lt B/E mangled limb	10
9	26	M	RTA	Rt B/K mangled limb with DNVD	11
10	23	M	RTA	Rt femur & both bone Leg open Grade IIIC	12
11	29	M	RTA	Rt mangled foot with Rt femur # open Grade IIIA	11
12	20	M	RTA	Rt B/k mangled limb with Rt supra pubic Rami #, pubic diastasis with sacroiliac Joint Disruption	13
13	70	M	RTA	Rt mangled foot with DNVD	11
14	40	M	RTA	Lt mangled lower Limb with DNVD	7
15	31	M	RTA	Rt mangled foot with Rt femur # open Grade II	7

MESS: Mangled extremity severity score, RTA: Road traffic accident, MOI: Mode of injury, B/K: Below knee, A/k: Above knee, B/E: Below elbow

Table 3: Hematoxylin and eosin stain results

Group I	Zone A (%)	Zone B (%)	Zone C (%)	*P*		
				Zone A versus B	Zone B versus C	Zone A versus C
Necrosis	100	86.60	40	0.5	0.016	0.004
Viability	6.70	20	73.30	0.5	0.008	0.002
Inflammation	40	73.30	60	0.063	0.5	0.37

Table 4: Statistical results of Immunohistochemistry staining with creatine Kinase MM antibody

Patients			Frequency	Mean	SD	Median	*P*		
							ZONE A-B	ZONE B-C	ZONE A-C
ZONE A	CK-MM	Pattern	15	2.93	0.258	3	0.102	0.004	0.004
		Intensity	15	2.93	0.258	3	0.18	0.008	0.004
		Combined	15	5.87	0.516	6	0.109	0.005	0.004
ZONE B	CK-MM	Pattern	15	2.67	0.724	3			
		Intensity	15	2.73	0.704	3			
		Combined	15	5.4	1.404	6			
ZONE C	CK-MM	Pattern	15	1.6	1.183	1			
		Intensity	15	1.8	0.941	1			
		Combined	15	3.4	2.098	2			

was also statistically significant with $P = 0.002$ and 0.008, respectively [Table 3].

Immunohistochemistry evaluation of creatine kinase-skeletal muscle

In Zone A, large muscle fascicles with complete loss of staining were noted with a median combined Score 6 with a range of 2–6. In Zone B, a large group of muscle fascicles with moderate to complete loss of staining were noted with a median combined Score 6 with a range of 2–6. In Zone C, muscle fibers with mild to moderate loss of staining were noted with combined Scores of 2 and 3, having a median Score of 2 [Figure 2a and b].

In Zone A and B, the median CK-MM score for both pattern and intensity was 3 with a range of 1–3, and for a combined score, it was 6 with a range of 2–6. Whereas for Zone C, it was 1 with a range of 1–3 for both pattern as well as the intensity, while the combined CK-MM score was 2 with a range of 2–6 respectively. On the application of the Wilcoxon signed-rank test, this decrease from Zone A to C and from Zone B to C was found statistically significant. This implies that the staining of CK-MM increased from Zone A to Zone C, while the CK-MM scores increase from Zone C to A [Table 4]. Further, IHC staining was noted to be more sensitive than H and E stains, as those myofibers that appeared normal on H and E sections showed some focal loss of staining and involvement of a few muscle fibrils.

Inference

CK-MM scores were higher in Zone A and lowest in Zone C. Higher scores were observed in those sections where a large group of fascicles had significant loss of CK-MM stains. These observations were corroborating with H and E stain findings. In Zone A (mangled part), a higher proportion of necrotic tissues corresponds to higher CK-MM scores, while in Zone C, as the proportion of viable muscle fibers increases, the CK-MM scores decrease.

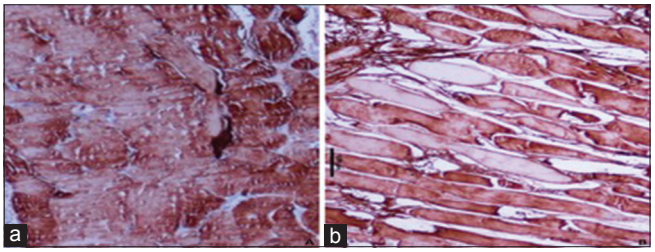

Figure 2: Photomicrographs showing the different intensity of immunohistochemistry staining for creatine kinase-MM in degenerated muscle, (a) injured muscle showing mild degree loss of creatine kinase-MM proteins by a large number of muscle fibers (peroxidase antiperoxidase, ×400), (b) injured muscle showing a total loss of staining for creatine kinase-MM protein by just a few scattered muscles (peroxidase antiperoxidase, ×400)

Discussion

In the present study, we were able to demonstrate a substantial increase in viable muscle fibers (6.7%–73%) from Zone A to C on the H and E examination [Table 3]. These observations further corroborated with observations of IHC staining with the CK-MM antibody. Using our arbitrary division into 3 zones, higher scores of CK-MM were noted in Zone A (with more percentage of nonviable fibers) compared to lower CK-MM scores in healthy muscle zone (Zone C) [Table 4]. Our preliminary data point toward CK-MM being a more sensitive marker for muscle viability compared to H and E staining alone; this allows the surgeon more insight into options of limb salvage when the decision is problematic.

Table 5: Overview of some studies related to creatine kinase-MM

Author	Study	Conclusion
Apple *et al*.	Profile of CK in skeletal muscle of marathon runner	Increased CK-MB, CK-BB, and mitochondrial CK activity in a biopsy of the gastrocnemius muscle of marathon runners
Jeong Gee Kim *et al*.	Clinical Significance of CK-MM Isoform Analysis for the Diagnosis of Neuromuscular Diseases	Tissue type CK-M is an excellent marker for diagnosis in the very early stage of AMI Total CK activity is a sensitive and excellent biochemical marker for the diagnosis of neuromuscular diseases
Szumilak *et al*.	Rhabdomyolysis: clinical features, causes, complications, and treatment	Increased level of CK in serum
Wolf *et al*.	Changes in serum enzymes, lactate, and haptoglobin following acute physical stress in international-class athletes	Total CK increased significantly after a training session
Brancaccio *et al*.	Serum enzyme monitoring in sports medicine	Serum CK level raised by damage to the muscle tissue due to intense prolonged training
Nigro G *et al*.	A prospective study of X-linked progressive muscular dystrophy in Campania	High level of serum CK in patients with myopathy

CK: Creatine kinase, AMI: Acute myocardial infarction

Sufficient evidence is recorded in the literature showing altered expression of serum CK-MM in the event of acute muscle insult, either in the form of direct or indirect injury.[5-17] Wolf *et al*. had demonstrated a rise in serum CK-MM levels, along with lactate, in association with acute physical stress in international athletes.[15] Similar findings were reported by Brancaccio *et al*. in 2008.[6] Most of the published literature has focused on serum CK activity, and only a few have studied the altered expression of CK in muscle tissue in the event of injury [Table 5]. Apple *et al*. subsequently studied the profile of CK-MM isoenzyme in skeletal muscles in marathon runners and found an increase in CK-MB, CK-BB, and mitochondrial CK, postmarathon running on skeletal muscle biopsy.[18] This naturally evolves into a thought process that brings into focus the correlation of this enzyme with acute skeletal muscle trauma, with the possibility of the predictive value of tissue viability. To date, no English literature has explored the role of tissue CK-MM in predicting muscle viability, particularly in mangled injuries [Table 1]. This study has been initiated as a pilot project to explore and validate the role and utility of tissue CK-MM as a biomarker in such high-velocity trauma.

Although our data point toward CK-MM staining having a potential role as a predictor of muscle viability and integrity, it is also important to note that those tissue sections that show normal morphology on H and E stains have shown the variable intensity of CK-MM loss on IHC staining. This probably indicates the high sensitivity of this biomarker in tissue for muscle viability and needs further research and corroboration in bigger patient subsets. Our preliminary work points toward the potential role of CK-MM in predicting the tissue salvageability, by delineating the nonviable tissue, but the clinical correlation is important.

The main limitations are the high cost and quantity of antibody, and the current study has a limitation of small sample size. Furthermore, the 2-part scoring system devised and used for CK-MM is unique and quite subjective. Larger studies, preferably multicentric, may give us more sound insights into this as a predictor of muscle viability in the future.

Conclusion

Preliminary data point toward CK-MM being a potential marker of muscle integrity, as it is a good indicator for identifying nonviable muscle; this may not be obvious in HE-stained sections. The advantage of CK-MM having differential expression in different zones of muscle injury alludes to its potential role as a predictor for amputation.

Recommendation

The study highlights the role of tissue CK-MM as a biomarker, by defining the extent of skeletal muscle trauma by delineating viable and nonviable muscle fibers. Large multicenter trials are recommended to consolidate these results and to further explore the clinical applicability of this biomarker in day-to-day practice. The current study is an attempt to widen the horizons for better understanding and management of mangled injuries in the nearby future.

Ethical consideration

This study was approved by the review board committee of the PGIMER, Chandigarh, India. The study strictly adhered to guidelines of the Indian Council of Medical Research (ICMR), 1994 and Helsinki declaration of 1975, revised in 2000.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Author's contribution

RKK, VK, and RK conceived and designed the study. KV and RK provided research material, collected, and organized data. KV and RK analyzed and interpreted the data. MSD, VK, and RK contributed to the definition of intellectual content and wrote initial and final drafts of the article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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