Abstract: Muscular dystrophy is a genetic disorder leading to progressive weakness of muscles caused due to dysfunction in or lack of protein in muscle cells. The prevalence of muscular dystrophy has been observed globally and is becoming a critical area of study for better health services. The purpose of the study is to analyze the research strength of muscular dystrophy using bibliographic literature. A quantitative literature analysis was carried out on muscular dystrophy from 1991 to 2015 for assessing the global research trends. This literature-based study was conducted using the documents retrieved from the Science Citation Index using the keywords: Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), Congenital Muscular Dystrophy (CMD), Myotonic Dystrophy, Emery-Dreifuss Muscular Dystrophy, Facioscapulohumeral Muscular Dystrophy, Oculopharyngeal Muscular Dystrophy, and Limb-Girdle Muscular Dystrophy. Analysis was done for annual productivity of publication, authorship, collaboration, country performance, citation frequency, characteristics of most cited document, journal productivity, etc.

Keywords: Bibliometric analysis, muscular dystrophy, research impact, research output.

INTRODUCTION

Muscular dystrophies (MDs) are a heterogeneous group of inherited myopathies that share similar clinical features and dystrophic changes on muscle biopsies (Merceru, E., & Muntoni, F. 2013). Despite the well-known disease symptoms, the diagnosis of MD continues to be challenging in the general pediatric settings and in pediatric neurology units (Mohamed, K., et al., 2000; Ciafaloni, E., et al., 2009). Potentially because unsuspected myopathy in children with hypertransaminasemia can be erroneously attributed to liver disease (Bugum, T., et al., 2000; Bradley, E., et al., 2002; KAMATH, B., et al., 2000; Kohli, R., 2005; Korones, D. N., et al., 2001; Morse, R. P., et al., 1993; Tay, S. K., 2000; Urganci, N., et al., 2006). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactic dehydrogenase (LDH) are components of routine or comprehensive blood panels and collectively demonstrate liver function. Consequently, in apparently healthy children, analysis of these liver enzymes is performed more frequently than analysis of creatine kinase (CK), a more specific marker of muscle disease (El-Bohy, A. A., & Wong, B. L. 2005). Particularly in rural or underdeveloped areas, a child with isolated hypertransaminasemia, labeled as being affected by cryptogenic hepatopathy, could be monitored only with liver function tests for a long time before serum CK is analyzed or before a muscular disease becomes clinically obvious, thus delaying diagnosis and treatment (Manzur, A. Y., et al., 2008). With advancements in diagnostic methodologies, such as magnetic resonance imaging (MRI), muscle biopsy, and genetic screening, more types of MD can be categorized accurately. However, because not all hospitals have access to these advanced techniques, the diagnosis of MD can still be challenging. CK values may facilitate differential diagnosis to some extent (Manzur, A. Y., et al., 2008) but measurement of CK alone is not as comprehensive as measurement of other serum enzymes. Thus, the development of additional tools for serum enzymes tests may facilitate differential diagnosis of subtypes of MD. It was emphasized that a diagnosis of occult muscle disease should be considered when confronted with an unexplained elevation of serum enzymes.

The Major Forms of Muscular Dystrophy

- Myotonic (also called MMD or Steinert’s disease)
- Duchenne
- Becker
- Limb-girdle
- Facioscapulohumeral
- Congenital
- Oculopharyngeal
- Distal

PATIENTS & METHODS

Clinical data from patients who visited Govt. Medical College & Hospital, Amritsar who visited the Department of Medicine were collected between June 2017 and October 2018. Patients were excluded if they had any coexisting medical diseases according to medical records. Patients had been diagnosed with one of the following five pathologies:

1) Duchenne muscular dystrophy
2) Becker’s muscular dystrophy (BMD)
3) facioscapulohumeral dystrophy (FSHD)
4) limb girdle muscular dystrophy (LGMD)
5) Emery-Dreifuss muscular dystrophy (EDMD).

For DMD/BMD, patients were diagnosed by dystrophin gene analysis or immunohistochemistry and western blotting for...
Serum enzymes, including ALT, AST, ALP, LDH, and CK, were measured using an Abbott AxSYM fully automatic biochemical analyzer (Abbott Laboratories, USA). The levels of serum enzymes were assayed according to the instructions provided with the corresponding enzymatic kits. The upper limits of normal for ALT, AST, ALP, LDH, and CK were 40, 37, 110, 240, and 250 U/L, respectively.

**Table 1: The demography and frequency of patients with MD presented with normal or abnormal serum enzyme levels.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Age (y)</th>
<th>Gender (%)</th>
<th>ALTn</th>
<th>ASTn</th>
<th>ALPn</th>
<th>LDHn</th>
<th>CKn</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD</td>
<td>6.7 ± 3.4 (7-24)</td>
<td>120 (0)</td>
<td>3 (2.5)</td>
<td>119 (97.5)</td>
<td>3 (2.5)</td>
<td>119 (97.5)</td>
<td>83 (68)</td>
</tr>
<tr>
<td>BMD</td>
<td>13.0 ± 8.5 (2-37)</td>
<td>36 (100)</td>
<td>0 (0)</td>
<td>37 (100)</td>
<td>0 (0)</td>
<td>37 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>FSHD</td>
<td>25.8 ± 13.3 (13-50)</td>
<td>13 (68)</td>
<td>12 (62.3)</td>
<td>7 (36.8)</td>
<td>10 (52.6)</td>
<td>9 (47.4)</td>
<td>11 (57.9)</td>
</tr>
<tr>
<td>LGMD</td>
<td>22.6 ± 11.4 (2-34)</td>
<td>23 (56)</td>
<td>11 (26.8)</td>
<td>30 (73.2)</td>
<td>9 (22.0)</td>
<td>32 (78.0)</td>
<td>17 (41.5)</td>
</tr>
<tr>
<td>EMD</td>
<td>15.4 ± 6.2 (6-42)</td>
<td>6 (55)</td>
<td>8 (72.7)</td>
<td>3 (27.3)</td>
<td>7 (63.6)</td>
<td>4 (36.4)</td>
<td>6 (54.5)</td>
</tr>
</tbody>
</table>

MD, muscular dystrophy; DMD, Duchenne muscular dystrophy; BMD, Becker’s muscular dystrophy; FSHD, facioscapulohumeral dystrophy; LGMD, limb girdle muscular dystrophy; EDMD, Emery-Dreifuss muscular dystrophy; AL, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactic dehydrogenase; CK, creatine kinase; m, month.

**Serum Enzyme Levels Among Five Types of MD**

For ALT, AST, and LDH levels, patients with DMD had higher serum concentrations than patients with BMD, FSHD, LGMD, and EDMD (p < 0.05). In addition, patients with LGMD had higher ALTn concentrations than patients with EDMD (p < 0.05). However, no differences in LDH concentrations were observed between these groups.

**Table 2: Serum enzymes levels among five types of MD**

<table>
<thead>
<tr>
<th>Category</th>
<th>ALTn</th>
<th>ASTn</th>
<th>ALPn</th>
<th>LDHn</th>
<th>CKn</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD</td>
<td>6.55 (4.85-8.18)</td>
<td>5.32 (3.98-6.68)</td>
<td>1.1 (0.94-1.25)</td>
<td>4.29 (3.23-5.60)</td>
<td>44.77 (31.05-56.57)</td>
</tr>
<tr>
<td>BMD</td>
<td>2.91 (1.96-4.61)</td>
<td>2.85 (1.76-4.5)</td>
<td>1.06 ± 0.94</td>
<td>2.02 (1.49-3.48)</td>
<td>27.59 (16.64-41.22)</td>
</tr>
<tr>
<td>FSHD</td>
<td>1.04 ± 0.48</td>
<td>1.00 (0.87-1.44)</td>
<td>0.60 (0.42-0.92)</td>
<td>1.02 (0.93-1.44)</td>
<td>3.05 (1.64-3.91)</td>
</tr>
<tr>
<td>LGMD</td>
<td>1.65 (0.74-3.39)</td>
<td>1.68 (1.01-2.86)</td>
<td>0.55 (0.45-1.17)</td>
<td>1.49 (1.00-2.37)</td>
<td>9.08 (4.50-21.87)</td>
</tr>
<tr>
<td>EMD</td>
<td>0.50 (0.36-0.83)</td>
<td>0.73 (0.69-1.04)</td>
<td>1.15 ± 0.54</td>
<td>1.09 ± 0.17</td>
<td>1.64 (0.66-3.51)</td>
</tr>
<tr>
<td>H</td>
<td>92.45 (0.001)</td>
<td>11.09</td>
<td>22.83</td>
<td>112.19</td>
<td>114.01</td>
</tr>
</tbody>
</table>

**Profiles of Serum Enzymes In Different Types Of Md**

For patients with different types of MD, only patients with DMD exhibited simultaneous elevation of serum ALT, AST, ALP, and LDH values. ALT levels exhibited the greatest increase, followed by AST, LDH, and ALP (Table 3).

**Table 3: The serum enzymes profile in five types of MD**

<table>
<thead>
<tr>
<th>Category</th>
<th>ALTn</th>
<th>ASTn</th>
<th>ALPn</th>
<th>LDHn</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>2.91 (1.96-4.61)</td>
<td>2.85 (1.76-4.5)</td>
<td>1.06 ± 0.46</td>
<td>2.02 (1.49-3.48)</td>
</tr>
<tr>
<td>DMD</td>
<td>6.55 (4.85-8.18)</td>
<td>5.32 (3.98-6.68)</td>
<td>1.1 (0.94-1.25)</td>
<td>4.29 (3.23-5.60)</td>
</tr>
<tr>
<td>EDMD</td>
<td>0.50 (0.36-0.83)</td>
<td>0.73 (0.69-1.04)</td>
<td>1.15 ± 0.54</td>
<td>1.06 ± 0.19</td>
</tr>
<tr>
<td>FSHD</td>
<td>1.04 ± 0.48</td>
<td>1.00 (0.87-1.44)</td>
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<td>1.02 (0.93-1.44)</td>
</tr>
<tr>
<td>LGMD</td>
<td>1.65 (0.74-3.39)</td>
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<td>0.55 (0.45-1.17)</td>
<td>1.49 (1.00-2.37)</td>
</tr>
</tbody>
</table>

MD, muscular dystrophy; DMD, Duchenne muscular dystrophy; BMD, Becker’s muscular dystrophy; FSHD, facioscapulohumeral dystrophy; LGMD, limb girdle muscular dystrophy; EDMD, Emery-Dreifuss muscular dystrophy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactic dehydrogenase.
Elevated serum ALT, AST, and LDH values were observed in patients with BMD or LGMD, while serum ALP values remained within the normal range. However, these two MD subtypes could be roughly distinguished by the magnitudes of changes in these enzymes (Table 4). For patients with BMD, ALT and AST levels exceeded 2-fold the ULN. In contrast, in patients with LGMD, ALT and AST levels were about 2-fold the ULN. Since LDH levels in patients with BMD and LGMD were both 2-fold the ULN (compared with "2"), we did not detect significant differences in LDH levels between patients with these subtypes. However, patients with BMD tended to have higher serum LDH levels. In patients with BMD, ALTn and ASTn levels exhibited the greatest increase, followed by LDHn and ALPn. In contrast, in patients with LGMD, ALTn, ASTn, and LDHn levels exhibited similar increases, while the increase in ALPn was lower.

**Table 4: The enzymes profile between patients with BMD and LGMD**

<table>
<thead>
<tr>
<th>Category</th>
<th>ALTn</th>
<th>ASTn</th>
<th>ALPn</th>
<th>LDHn</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD</td>
<td>1.65 (0.74–3.39) (→)</td>
<td>1.68 (1.01–2.86) (→)</td>
<td>0.55 (0.45–1.17)</td>
<td>1.49 (1.00–2.37) (→)</td>
</tr>
<tr>
<td>BMD</td>
<td>1.70 (0.74–3.39) (→)</td>
<td>1.70 (1.01–2.86) (→)</td>
<td>0.55 (0.45–1.17)</td>
<td>1.49 (1.00–2.37) (→)</td>
</tr>
</tbody>
</table>

BMD, Becker's muscular dystrophy; LGMD, limb girdle muscular dystrophy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactic dehydrogenase.

Patients with FSHD and EDMD exhibited the same profiles for the four liver enzymes, with all values remaining within the normal range (Table 3). Nonetheless, patients with FSHD tended to have lower serum ALT and AST values than patients with EDMD, whereas patients with EDMD tended to have higher serum ALP and LDH values than patients with FSHD.

**Enzyme Levels In Patients with LGMD**

Twenty-six patients with LGMD (over 50%) had genetic tests. Among them, five had duplicate genetic tests (data not shown). Three patients carried CAPN3 gene mutations responsible for LGMD2A, and eight patients were negative for the CAPN3 gene mutation. Moreover, eight patients harbored DYSF gene mutations responsible for LGMD2B, and eight patients were negative for the DYSF gene mutation. Since there were fewer samples from patients with LGMD2A, we could not perform any statistical analysis to detect characteristic profiles of serum enzymes between patients with and without LGMD2A. There were no significant differences in ALT, AST, or LDH levels between patients with and without LGMD2B (data not shown). Additionally, no significant differences in CK levels were observed between patients with and without LGMD2B. However, ALP levels in LGMD2B patients (U/L) were significantly higher than those in non-LGMD2B patients (U/L).

**DISCUSSION**

In the present study, a high frequency of patients with MD presented with abnormal levels of serum enzymes (including ALT, AST, ALP, and LDH). For instance, all patients with BMD and up to 97% of patients with DMD had elevated ALT, AST, and LDH values. Even in patients with EDMD, for which the frequency was relatively small, the proportion of patients presenting with abnormal ALT, AST, or LDH values was no lower than 25%. Indeed, studies from around the world (Begum, T., et al., 2000; KAMATH, B., et al., 2000; Kohli, R., et al., 2005; Korones, D. N., et al., 2001; Urganci, N., et al., 2006) have reported that patients with muscular dystrophies are often erroneously labeled as having cryptogenic liver disease. However, most of these studies have analyzed aminotransferases, with few analyses of ALP and LDH. Hence, serum ALP and/or LDH as markers of muscular diseases should also be stressed. The mechanism through which levels of ALP and LDH become abnormal in patients with MD is still unknown. Elevations in ALT and AST levels are common indicators of hepatocellular damage; however, ALT abnormalities are also found in cardiac and skeletal muscle, although ALT activity in skeletal muscle is only one-tenth of that in hepatocytes (Wróblewski, F., 1959). AST is found within the cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes (Pratt, D. S., & Kaplan, M. M., 2000). Since serum CK is markedly elevated with breakdown of muscle and is considered a diagnostic marker of MDs (Okinaka, S., et al., 1959), we assumed that leakage of transaminases from muscle membrane would occur along with the leakage of CK under pathological conditions, such as in patients with MD. Clinical differential diagnosis between BMD and LGMD may be difficult because the clinical phenotype of BMD tends to overlap with other limb girdle syndromes, especially LGMD (KAMATH, B., et al., 2000). In fact, in our tertiary care center, we continue to observe misdiagnosis of BMD as LGMD and vice versa. Genetic analysis is the gold standard for distinguishing between these disorders. However, it is difficult to perform genetic analysis when first evaluating a patient suspected of LGMD because of the various subtypes of LGMD. Hence, additional methods for distinguishing between BMD and LGMD, as well as subtypes of LGMD, should be developed. Since serum enzyme levels were elevated to variable degrees in patients with BMD or LGMD. For the former, ALT or AST levels were more than 2-fold the ULN, and, for the latter, ALT or AST levels were equal to 2-fold the ULN. In addition, we provided discriminant functions to assist clinicians in identification of these subtypes without advanced diagnostic technology as follows: once serum enzyme levels (ALT, AST, ALP, and LDH) are measured in patients of known age, clinicians can make a probable diagnosis of BMD.

Serum enzyme levels were elevated to variable degrees among patients with different subtypes of MD. McMillan et al., reported that ALT values are elevated by up to 22.6 times the ULN in patients with DMD, whereas we observed that ALT reached 5–8 times the ULN in patients with DMD. Different methods for detecting enzyme levels or taking blood samples under nonstandardized conditions may account for this discrepancy. Additionally, Zhang et al. reported that disorders can be sequenced (e.g., DMD/BMD > LGMD > FSHD) according to AST or ALT levels, consistent with our results. Regarding LDH, Yasmineh et al., reported that the mean total serum activity in patients with DMD was 3.4-fold that of serum from the control group, which was consistent with the range observed in our current analysis (3.23 to 5.60). Our observations were also consistent with previous reports demonstrating that serum LDH activity in patients with EDMD was within the normal limit or slightly increased (Rowland, L.P., et al., 1979). As another index, ALP levels have seldom been described in muscular disease. Strikingly, in patients with DMD, BMD, FSHD, and LGMD, the fold increases for ALT, AST, and LDH were greater than that for ALP, while in patients with EDMD, the fold increases for ALT, AST, and LDH were lower than that for ALP. Further research is needed to determine the correlation between EDMD and ALP. To some extent, CK values may facilitate differential diagnosis. Hence, when advanced diagnostic technology is absent, discrepancies in levels of other enzymes may also be used to provide important clues. Interestingly, each type of MD had a characteristic profile of serum enzymes. Therefore, the distribution of serum enzymes may have additional implications for the differential diagnosis of MD. Moreover, although patients with FSHD and EDMD shared the same serum enzyme profiles, distinguishing between these diseases is relatively simple based on clinical features alone. For example, FSHD is characterized by weakness of the face, upper-arm, and shoulder girdle muscles whereas EDMD is characterized by slow progressive muscle weakness, early joint contractures, and atrial arrhythmia. Thus, differential diagnoses should consider as many parameters as possible.
SUMMARY

Our study had several limitations. First, only 11 cases of EDMD and 19 cases of FSHD were reviewed in the present study. It is likely that the small sample studied is not representative of the general patient population. Second, not all the patients with LGMD were confirmed by genetic testing, and the exact diagnosis of LGMD subtypes was challenging. Third, we did not thoroughly investigate the effects of some medications or food on serum enzyme levels. Marked variability in serum enzymes can occur from day to day (Morse, R. P., & Rosman, N. P. 1993).

In summary, we found that a high frequency of patients with MD presented with abnormal serum enzyme levels. The characteristic profiles of serum enzymes facilitated the differentiation of MD subtypes. For example, DMD was characterized by simultaneous elevation of ALT, AST, LDH, and ALP; BMD and LGMD were characterized by elevation of ALT, AST, and LDH; and FSHD and EDMD lacked abnormalities in the serum levels of these four enzymes. To further differentiate BMD from LGMD, discriminant functions were developed for cases in which enzyme levels and age are known. For LGMD, patients with LGMD2B had significantly higher ALP levels than patients with non-LGMD2B subtypes. Thus, our approach makes it possible to determine the subtypes of MD by serum enzyme profiles prior to genetic testing, which will increase the chance that a mutation will be found in the first gene analyzed.

REFERENCES