The Impact of Chronic Liver Diseases on the Level of Heart-Type Fatty Acid-Binding Protein (H-FABP) Concentrations

Hafidh A Al-Hadi,1 Brent William,2 Keith A Fox1

Abstract: Objectives: Heart-type fatty acid binding-protein (H-FABP) has been reported to be a potential novel biochemical marker for the early diagnosis of acute myocardial infarction (AMI). The presence of H-FABP in the blood has not been reported. The aim of this study was to compare the effect of chronic liver diseases on the level of H-FABP concentrations. Methods: The effects of chronic liver diseases including infective hepatitis and cirrhosis on the concentration of H-FABP was studied in a small group of patients (n=10, mean age ± SD = 58.33 ± 7.19 years). The serum concentrations of the following markers were measured: H-FABP, alanine aminotransferase (ALT) and bilirubin and compared with a reference control group (20 healthy blood donors, mean age ± SD = 63.8 ± 8.01). Results: The serum concentrations of these markers in the control group as compared to patients with chronic liver disease were as follows (mean ± SD): H-FABP = 6.86 ± 2.21 µg/L versus 6.44 ± 3.06 µg/L (p > 0.0001); ALT = 29.8 ± 14.7 U/L versus ALT = 198.67 ± 122.89 U/L (p < 0.0005) and bilirubin = 9.6 ± 4.0 µmol/L versus bilirubin = 100.89 ± 87.85 µmol/L (p < 0.0001). Conclusion: These data illustrate clearly that there is no significant interference with the normal concentration of H-FABP in the presence of liver diseases, despite the significant elevation of liver enzymes and proteins. These data may support a useful role of H-FABP for the diagnosis of myocardial injury in patients with liver diseases.

Keywords: Heart-type fatty acid binding protein (H-FABP); Chronic liver diseases; Bilirubin; Alanine aminotransferase.

Advances in Knowledge
1. Heart-type fatty acid-binding protein is a useful early marker for the diagnosis of acute myocardial infarction.
2. The effect of chronic liver diseases on the diagnostic potential of this marker is not known.
3. This article illustrates the lack of interferences of the various types of chronic liver diseases on the ability to use heart-type fatty acid binding protein as an early cardiac marker for the early diagnosis of acute myocardial infarction.

Application to Patient Care
1. The information provided in this article will help health institutions caring for patients with acute myocardial infarction on how best to use, interpret and apply the results obtained with heart-type fatty acid-binding protein in patients presenting with acute chest pain suggestive of evolving acute myocardial infarction who also have various co-existing types of chronic liver diseases.

1Department of Medicine, Sultan Qaboos University Hospital, Muscat, Sultanate of Oman. 2Department of Medical Sciences, Faculty of Medicine, University of Edinburgh, UK. *University of Edinburgh, Cardiovascular Research Unit, Royal Infirmary of Edinburgh, UK.

*To whom correspondence should be addressed. Email: halhadi@hotmail.com
**Heart-type fatty acid binding-protein (H-FABP)** is a small soluble non-enzyme protein composed of 132 amino acids. It is one of the most abundant proteins in the heart and comprises 5-15% of the total cytosolic protein pool. H-FABP exists in high concentrations in the heart; however, this protein is not totally cardiac specific and occurs in other tissues although in a much lesser concentrations.

H-FABP was introduced by Glatz et al. in 1988 as a potential novel biochemical marker for the early diagnosis of acute myocardial infarction (AMI). This was soon confirmed in many other studies. Some of the more recent studies have questioned the value of these early markers (H-FABP and myoglobin) when compared with specific markers like cardiac troponin I (cTnI).

H-FABP is released into plasma within 2 hours after symptom onset and is reported to peak at about 4-6 hours and return to normal base line value in 20 hours. Within the period of 30-210 minutes after symptom onset, H-FABP has > 80% sensitivity for the diagnosis of AMI. Within the interval of 0-6 hours after symptom onset, the other cardiac markers such as creatine kinase (CK), CK-MB mass or activity, cTnI and T (cTnT) will only be starting to accumulate in the plasma, and their sensitivity has been reported to be around 64%.

The exact route(s) of excretion of H-FABP from the circulation is not fully understood. As suggested by previous studies, the kidney may be the major route of excretion of H-FABP from circulation. A rise in serum and urine H-FABP concentration above normal values is seen in patients who present with AMI as early as 1.5 hours after symptom onset.

The presence of H-FABP in the liver has not been reported. However, an isoform specific to the liver called liver-type FABP exists. The interferences of this protein and chronic liver diseases on the concentration of H-FABP has not been studied before. Also, the effect of chronic liver diseases on the release of H-FABP from other tissues has not yet been fully evaluated. Therefore, the aim of the study was to compare the effect of chronic liver diseases on the serum levels of H-FABP.

### Methods

The effects of disease states in particular chronic liver diseases on the normal concentration of H-FABP was studied in 2003-2004 a small group of patients with a mixture of chronic liver disorders (n=10, mean age ±SD = 58.33 ±7.19 years, range 45-70 years, median = 59 years) These patients had a range of conditions including infective hepatitis and cirrhosis (chronic hepatitis B = 2, chronic hepatitis C = 2, chronic alcoholic hepatitis = 3, other cirrhosis = 3). They were recruited from the Liver Unit at the Edinburgh Royal Infirmary, UK. Ethical approval was obtained from the local ethical committee (Lothian Research Ethics Committee, Edinburgh) and informed consent was obtained from each patient before beginning the study. The study complies with the Declaration of Helsinki. The serum concentrations of the following markers H-FABP, ALT and bilirubin were measured in the study group and compared with the concentrations of these markers in a normal reference control group of healthy blood donor controls (n=20, mean age ±SD = 63.8 ±8.01, range 53-75 years, median = 65 years). Peripheral blood samples for serum analysis were collected in white Starstedt Monovette vacutainer tubes by venepuncture. The blood samples (5mls) were taken through a peripheral line (intravascular access). The extracted samples were allowed to clot at room temperature for 1 hour and then centrifuged at 4°C, and the resulting serum was divided into small aliquots and frozen at -70°C until analysis. H-FABP was analysed by an enzyme linked immunosorbent assay method using commercially available assays (Hycult, Cambridge). Bilirubin and ALT were measured in the Biochemistry Department of the Edinburgh Royal Infirmary on an automated analyser machine using commercial assays.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS™, Pittsburgh, Version 15). Continuous variables were presented as mean ± standard deviation. Comparisons between the study group and control group variables were conducted by the Mann-Whitney U test for continuous variables. Significant results were indicated by probability values less than or equal to 0.05.
Results

The analytical sensitivity of H-FABP assay (mean ± 2SD) was 0.206 ±0.047 g/L.17 The normal concentrations of these markers in the normal reference control group of healthy blood donor controls were as follows: H-FABP = 6.86 ±2.21 µg/L; ALT = 29.8 ±14.7 U/L and bilirubin = 9.6 ±4.0 µmol/L. The study group consisted of 10 patients. The concentration of these markers in patients with chronic liver diseases were as follows: H-FABP = 6.44 ±3.06 µg/L, (range 2-11 µg/L, median = 7 µg/L); ALT = 198.67 ±122.9 U/L, (range 73-500 U/L, median = 114 U/L) and bilirubin = 100.89 ±87.85 µmol/L, (range 17 - 337 µmol/L, median = 66 µmol/L). There was no significant difference between the concentration of H-FABP in the study group and controls; however, the concentrations of liver enzymes and protein (ALT and Bilirubin) were significantly elevated in the study group [Table 1].

Discussion

Under normal conditions H-FABP is present in plasma in very low concentrations (< 5 µg/L), but it is significantly elevated upon cellular injury.18 This makes the plasma estimation of H-FABP suitable for the early detection and quantification of myocardial tissue injury. However, this protein is not totally cardiac specific as it occurs in skeletal muscle in concentrations varying between 0.05-0.2 mg/g wet weight of tissue, depending on muscle fibre type studied.5 It has also been reported in very low concentrations in tissues like the kidney, aorta, testes, mammary glands, placenta, brain, adrenal glands, adipose tissue, and stomach.23 The concentration of H-FABP in the study group was not statistically different from the control group. This finding leads to several assumptions. First, the Liver-FABP (L-FABP) is a separate factor with no or negligible cross-reactivity with H-FABP assays. Indeed, the cross-reactivity between these two proteins has been reported to be < 0.005.17 Second, the release of H-FABP from other tissues containing this protein (see above) is at best minimal in patients who have chronic liver diseases. Our study was the first of its kind to address the interference of chronic liver diseases on the normal concentrations of H-FABP. There are no data on this issue in the literature hence it is difficult to correlate our findings.

In a previous study, we have shown major limitations for the use of H-FABP concentration for the diagnosis of myocardial injury in the presence of renal failure.19 The liver contains only L-FABP, but co-expression of H-FABP and L-FABP occurs in the kidney. Similarly, intestinal-type FABP (I-FABP) and L-FABP are found in intestines, and brain-type FABP (B-FABP) and H-FABP occur in the brain. Preliminary but promising applications of these proteins have been demonstrated for liver rejection, viability selection of kidneys from non-heart-beating donors (NHBD), inflammatory and ischaemic bowel disease, traumatic brain injury and in the prevention of muscle injury in trained athletes.20 Measurement of H-FABP in the first 24 hours after onset of symptoms may be potentially useful for the diagnosis of AMI; identification of patients who need reperfusion treatment early; identification of patients who reperfuse their infarct related artery; detection of re-infarction if it occurs early, and estimation of infarct size.21

Conclusion

These data illustrate clearly that there is no significant interference with the normal concentration of H-FABP in the presence of chronic liver diseases, despite the significant elevation of liver enzymes and proteins. This is consistent with the reduced cross-reactivity between H-FABP and other FABP.

Table 1: Age and concentrations of marker proteins in the control and study groups

<table>
<thead>
<tr>
<th></th>
<th>Control Group (Blood donors)</th>
<th>Study Group (Liver disease patients)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>n=20</td>
<td>n=10</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>63.8 ±8.0</td>
<td>58.33 ±7.2</td>
<td>(NS)</td>
</tr>
<tr>
<td>ALT</td>
<td>29.8 ±14.7</td>
<td>198.67 ±122.9</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>9.6 ±4.0</td>
<td>100.89 ±87.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>H-FABP</td>
<td>6.86 ±2.2</td>
<td>6.44 ±3.1</td>
<td>(NS)</td>
</tr>
</tbody>
</table>

Legend: NS = not significant; ALT = alanine aminotransferase; H-FABP = Heart-type fatty acid binding protein
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including L-FABP. These findings may support a useful role of H-FABP for the diagnosis of myocardial injury in patients with chronic liver diseases.

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The authors report no conflict of interest.

References

A Female Child with Skin Lesions and Seizures
Case report of Incontinentia Pigmenti

Sana Al-Zuhaibi,¹ Anuradha Ganesh,¹ Ahmed Al-Waili,³ Faisal Al-Azri,⁴ Hashim Javad,² *Amna Al-Futaisi ²

CASE REPORT

A Female Child with Skin Lesions and Seizures
Case report of Incontinentia Pigmenti

Sana Al-Zuhaibi,¹ Anuradha Ganesh,¹ Ahmed Al-Waili,³ Faisal Al-Azri,⁴ Hashim Javad,² *Amna Al-Futaisi ²

ABSTRACT: Incontinentia Pigmenti (IP), (OMIM # 308300), is a rare X-linked dominant condition. It is a multisystemic disease with neurocutaneous findings involving the skin, eyes, hair, nails, teeth, and central nervous system. It is usually lethal in males; the disease has variable expression in an affected female. We report the case of a 6 month old girl who presented at Sultan Qaboos University Hospital, Oman, with neonatal seizures and hypopigmented/hyperpigmented skin lesions. She had multiple ophthalmic abnormalities and neurological manifestations which are discussed in this report.

Keywords: Incontinentia Pigmenti (IP); Seizures; Ophthalmic; Hypomelanosis of Ito; Neurologic diseases; Case report; Oman.

Incontinentia Pigmenti (IP) Type 2, also known as Bloch-Sulzberger syndrome, is an inherited multisystem neurocutaneous disorder with a low incidence (1% of all neurocutaneous disorders).¹ It is an X-linked dominant condition. The disease is lethal in males, except in rare cases of somatic mosaicism, or mutations in IKBKG, and when the condition occurs in patients with Klinefelter syndrome.²³ Typical skin lesions are seen in 100% of (IP) patients.² Other manifestations include dental (90%), skeletal (40%), central nervous system (CNS) (40-50%) and ocular (35%) conditions.⁴⁵

Patients with IP frequently have systemic involvement, similar to the involvement in patients with hypomelanosis of Ito, including CNS manifestations. In patients with IP, cutaneous lesions undergo three stages, which may overlap.⁶

In this report, we discuss the dermatologic, neurological and ophthalmologic findings of a 6 month old female who presented with early onset neonatal seizures and displayed hypopigmented/hyperpigmented skin lesions. In addition, this child had characteristic ophthalmologic findings.

Case Report

This 6 month old girl was born by spontaneous vaginal delivery to healthy non-consanguineous parents with two normal sons. She was referred to Sultan Qaboos University Hospital (SQUH), Oman, from a peripheral hospital for evaluation of abnormal skin lesions and seizures.

She was reported to have had hyper/hypopigmented skin lesions all over her body except the face since she was 6 days old. The skin lesions were noted to be of linear pattern over the upper and lower limbs and of a whorled pattern over the anterior and posterior chest wall. The mother denied the presence of any skin lesion at birth. Her examination showed evidence of hypotonia and
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mild developmental delay with microcephaly where her head circumferences was significantly below the third percentile for age. Her weight was below the third percentile, but her height was normal for age. Her tone was mildly decreased with normal reflexes and positive Babinski reflex. She had a normal visual following, but was not able to respond to sound clinically. Other systemic examinations were normal.

The child developed clonic seizures at the age of 3.5 months and was commenced on oral phenobarbitone with good seizure control. The computed tomography (CT) scan of the brain (performed at a peripheral hospital) showed atrophy of the frontal horns with corpus callosum agenesis. A chromosomal study showed a normal female karyotype.

At SQUH, a magnetic resonance imaging (MRI) of the brain showed hypoplasia of the corpus callosum and hypomyelination with periventricular white matter hyperintense signal abnormalities [Figures 1a and 1b]. Electrophysiological testing showed positive visual evoked potential (VEP) responses using a flash stimulation, but negative responses for brain stem auditory evoked potentials (BAEP).

An ophthalmologic evaluation was performed at the age of 5 months. The child was able to follow and fixate with both eyes. There was no obvious nystagmus, ocular deviation or ptosis. Further examination under anaesthesia revealed bilateral inferior superficial epithelial corneal erosions; there were no stromal infiltrates or any increase in corneal thickness. The corneal size was normal for age in both eyes. She had a bilateral mild form of persistent pupillary membrane. She had a normal red reflex and normal reacting pupils with no evidence of any relative afferent defect. The intraocular pressure was 24mmHg in both eyes. No major refractive error was noted. A dilated fundus examination showed a large optic disc cup (disc cup ratio of 0.8 in both eyes). There were diffuse non-specific retinal pigment epithelial changes. The peripheral retinal examination revealed areas of fibrovascular proliferation with no evidence of retinal traction or detachment [Figures 2a and 2b]. The child was started on latanoprost 0.05% eye drops q.h.s. in both eyes with regular follow-ups at the eye clinic. Histological studies of the skin lesions and genetic studies were scheduled; unfortunately, the child died from status epilepticus at a peripheral hospital before these studies could be undertaken.

Discussion

IP is characterized by abnormalities of the tissues and organs embryologically derived from ectoderm and neuroectoderm. The diagnosis of IP is made on
clinical grounds aided by histological confirmation. The skin lesions may follow the Blaschko lines and the initial appearance of skin lesions can be seen immediately after birth or early during the neonatal period. Timely recognition of IP by pediatricians and dermatologists is therefore crucial. IP overlaps with hypomelanosis of Ito, which is a syndrome with hypopigmented whorls of the skin along the Blaschko lines, especially when it presents at the stage of skin hypopigmentation. Chromosomal mosaicism is believed to be the reason that hypomelanosis of Ito is so varied in phenotype. Certain genes, namely, those on 9q33-qter, 15q11-q13, and Xp11, have been implicated in hypomelanosis of Ito; however, no consensus exists about the identity of the hypomelanosis of Ito gene.5, 6

In around 40-50% of IP cases, there may be neurological problems such as seizures, spasticity, or mental retardation.1 Seizure can be the first manifestation of the disease.2 Abnormal tooth eruption, malformed tooth crowns, and patchy alopecia are commonly seen. Retinal dysplasia can sometimes lead to visual problems.

The diagnosis of IP is also aided by family history and a history of miscarriages of the male gender as the disease is prenatally lethal in males. The disease is caused by a genomic rearrangement of the gene for NEMO, or nuclear factor kappa B essential modulator (IKBKG-IKK gamma). The defect in the X chromosome is proximal to the gene for factor VIII at Xq28.7, 8 Although this child was the first affected in her family with two normal male siblings and no previous miscarriages in the family, she had hypopigmented/hyperpigmented lesions suggestive of the disease. Initially, the diagnosis was not entertained, despite the skin manifestations, until her presentation with partial seizures. The possibility of hypomelanosis of Ito diagnosis was also kept in mind though further evidence of neurological involvement and the eye manifestations were suggestive of IP.

The skin lesions in IP manifest in stages that evolve sequentially.7 The onset and duration of each stage vary among individuals; not all individuals experience all four stages.1 Typically four dermatological stages are seen: 1) the bullous stage; early blistering with eosinophilia; 2) the verrucous stage: eruption of hyperkeratotic lesions; 3) the hyperpigmentation stage: hyperpigmentation along the lines of Blaschko and, finally, 4) the atretic stage: dermal scarring. In the vast majority of cases, the onset of skin changes is before 6 weeks of age.1 Our patient had the third stage which was not preceded by the first or second stages. The differential diagnosis of patients presenting with stage 3 and 4 skin lesions includes: hypomelanosis of Ito; IP achromians; focal dermal hypoplasia syndrome (Goltz syndrome) and X-linked dominant chondrodysplasia punctata.

In IP, variable clinical expressions of CNS involvement are seen. They include epilepsy, mental retardation, hemiparesis, spasticity, microcephaly, and cerebellar ataxia.5 The pathogenesis of the CNS lesion in IP remains unclear.9,10,11 Recent brain-imaging techniques, such as MRI and magnetic resonance angiogram (MRA), have provided a better understanding of the nature of the CNS pathology. These imaging studies have demonstrated scattered cortical neuronal and white-matter necrosis,
hypoplasia of the corpus callosum, periventricular white-matter cystic lesions, neuronal heterotopia, and cerebral atrophy.12, 13

Our patient had evidence of hypoplasia of the corpus callosum and decreased myelination of the white matter and, clinically, both seizures and developmental delay. The presence of CNS involvement, such as seizures, in the neonatal period is a poor prognostic sign.6, 6, 7

Ocular abnormalities occur in 35% or more of patients and 19% are at risk of severe visual loss in one or both eyes.14,15 A wide range of ophthalmologic findings seen in patients with IP?7 The commonest reported are strabismus in 18.2% and a retrolental mass or retinal pseudoglioma in 15.4%.

There are previous reported cases in literature of multiple corneal abnormalities including megalocornea, corneal oedema, band keratopathy, bullus keratopathy, variable corneal epithelial and stromal changes and iridocorneal attachments.10 The corneal findings in this child were superficial punctate epithelial erosion and features suggestive of an inflammatory noninfectious process.

The posterior segment findings may include multiple retinovascular abnormalities (such as retinal vascular tortuosity, macular capillary dropout, peripheral arteriovenous shunts, retinal neovascularisation, and vitreous haemorrhage). Subsequently, preretinal fibrosis, pseudoglioma and traction retinal detachment can result.14 This child had findings suggestive of what looked like pseudogliomas in both eyes [Figures 2a and 2b] and peripheral fibrovascular lesions OU with no evidence of retinal traction.

Holstrom proposed a scheme for following patients with IP and eye manifestations. They recommended that eyes should be examined soon after birth, and then at least monthly for three to four months, at three-month intervals for one year, and twice yearly up to three years. They also recommended that the frequency of examinations should be increased in children with retinal disease. If, at three years of age, no abnormalities, refractive errors or strabismus are found, they state that the follow-up can cease.16

During the ophthalmic follow-up, visual functions should be assessed by both clinical and electrophysiological measures including VEP and electroretinography (ERG).16,17 Any significant refractive errors should be corrected and amblyopia therapy as well as strabismus treatment should be provided.18,19 Cryotherapy or laser photocoagulation should be applied for active peripheral retinal abnormalities and tractional retinal detachment should be treated surgically.

Conclusion
In summary, IP or Bloch-Sulzberger syndrome is a rare X-linked dominant syndrome. It has multisystemic involvement that includes the skin, central nervous system and eyes. In neurocutaneous syndromes, multidisciplinary care with periodic consultations with a paediatric ophthalmologist, neurologist and other specialists depending on the associated anomalies are essential. The differentiation between hypomelanosis of Ito and IP can be difficult as the two disorders overlap considerably. Clinicians need to be aware of the variable manifestations of this disease for a timely and multidisciplinary management of such patients.

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References


