HAEMOPHILIA B DIAGNOSTIC DISCREPANCY: A SURVEY TO CONFIRM THE DIAGNOSIS OF HAEMOPHILIA B

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ABSTRACT

OBJECTIVES: To determine the frequency of misdiagnosis among documented factor IX (FIX) deficient (hemophilia B) patients of Khyber Pakhtunkhwa (KP) province of Pakistan and to discern role of different diagnostic tests employed in this regard.

METHODS: Patients were identified through registries at transfusion centers. Out of 83 FIX deficient patients registered at transfusion centers and hemophilia care centers of KP province, 32 patients could be contacted and only 30 patients consented to be enrolled into the study. A comprehensive questionnaire depicting demographic details, clinical history and physical examination was filled out for each enrolled patient. Their blood samples were posed to first and second line coagulation tests both manually and on two different automated coagulation analyzers.

RESULTS: The registered FIX deficient patients’ pool was found to have a mean age of 17.39±9.8 years. Out of 30 enrolled patients, diagnosis was correct in 18 (60.0%) patients and wrong in 12 (40.0%) patients. The misdiagnosed cases were hemophilia-A (n=9; 30%) and hypo-fibrinogenemia (n=3; 10.0%). All cases diagnosed previously on the basis of factor assays (n=8, 100%) were found to be correct while those diagnosed previously with mixing studies, only 08/18 (44.44%) had FIX deficiency. Out of 4 cases diagnosed on circumstantial evidence of isolated prolonged APTT along with established FIX deficiency in another sibling or maternal cousin, 2 had FIX deficiency.

CONCLUSION: A large fraction of patients documented as FIX deficient on the basis of mixing studies are misdiagnosed. In clinical practice factor assays are more valid in comparison to mixing studies.

KEY WORDS: Hemophilia B (MeSH), Factor IX Deficiency (Non-MeSH), Diagnostic Discrepancy (Non-MeSH), Mixing Studies (Non-MeSH).

INTRODUCTION

Factor IX (FIX) deficiency, also called hemophilia B (HB) or Christmas disease, is an X-linked genetic disorder that causes deficiency of FIX of variable penetrance. Owing to the better management and increased survival rate of patients, a higher prevalence rate of 2.69±1.61 (mean ± SD) per 100,000 males is found in the high income countries; highest being 8.07 per 100,000 males in Ireland.1 Prevalence in rest of the world is 1.20±1.33 (mean ± SD) per 100,000 males.1 Prevalence of the disease in general population of Pakistan is unknown. In Iran, the neighboring country, a prevalence of 2.01 per 100,000 males has been found.2

Clinically FIX deficiency has been categorized as severe (FIX level: <1%), moderate (FIX level: 1-5%) and mild (FIX level: 6-40%).3 Surveys have shown that the incidence of severe class is highest (50%), followed by moderate (30%) and mild (20%) categories. Patients with severe hemophilia have short survival rate.4

As for most of other diseases, accurate diagnosis is essential for appropriate management of FIX deficiency. Definitive diagnosis of the disease is made by FIX: C level assessment by standard coagulation assays.4 Diagnosis of hemophilia in most of the concerned laboratories of Khyber Pakhtunkhwa (KP) province of Pakistan, however; is still based on findings of mixing studies. Primary reagents for this test, like aged and adsorbed plasma, are usually prepared in the testing laboratory. The test profile includes PT, APTT, testing patient plasma with normal pooled plasma, testing with adsorbed plasma, testing with aged plasma/serum. Further to this, the test accuracy and reliability depends on laborious bench work of manual handling and expertise of the test performer. All these factors render the test prone to flaws at several levels. Hemophilia is a debilitating disease with several adverse factors in play to debilitate the patient.5-6 Misdiagnosis is deemed to be an adverse prognostic factor.

In clinical practice laboratory test errors comprise 46-68.2% pre-analytical errors, 18.5-47% post-analytical
errors and 7-13% analytical errors. The frequency of error ranges from 1/330-1000 events or 1/900-2074 patients or 214-8316 laboratory results. Discreant assessments of factor VIII (FVIII) levels by one stage and chromogenic assays have been documented by several hemophilia centers worldwide. These studies focus FVIII levels; however; the newer FIX concentrates also have similar issues. Discrepent hemophilia diagnosis based on mixing studies, however; has not been reported elsewhere.

A study recently conducted on FVIII deficient population of Pakistan reported about 8% cases of misdiagnosis. This reciprocally, aroused suspicion of misdiagnosis in FIX deficient patients. Hence, the current study was conducted to estimate frequency of misdiagnosis in documented FIX deficient patients of KP province of Pakistan.

**METHODS**

**Study Design**

Our study had a descriptive, case-only study design with cross-sectional time prospect. All the procedures carried out were in accordance with the Helsinki Declaration of 1975, as revised in 1983. The study was approved by ethical committees of participating centers.

**Patients**

Patients possessing a laboratory test report depicting FIX deficiency were enrolled into the study. Patients with unclear diagnoses or those with major co-morbidities were excluded. In the initial part of the study, all pediatrics wards of major teaching hospitals, clinical laboratories and transfusion centers of the province and neighboring cities were surveyed in quest of FIX deficient patients belonging to the province KP. After acquisition of contact details of HB patients from the identified centers, these were contacted and voluntarily were enrolled into the study after acquiring due consent in writing. To avoid selection bias, all available registered FIX deficient patients were asked to volunteer for the study.

**Disease Assessment**

A comprehensive questionnaire was designed to record the bio-data, family history, pedigree, clinical history and physical examination of the patient. Exclusion of co-morbidities and assessment of clinical severity of the disease were also covered in the questionnaire.

**Blood Sample Collection and Processing**

A blood sample collection kit was prepared for each patient. Before taking blood sample, identity of the patient was established and it was confirmed that the individual had not received any factor concentrate, fresh frozen plasma (FFP) or any other blood product in the last two weeks. A volume of 3ml blood was collected aseptically in Na-citrate containing BD Vacutainer. Plasma was separated by centrifuging the blood at 1700 g for at least 10 minutes at room temperature. The supernatant plasma, so extracted, was put into aliquots of 1 ml Eppendorf® tubes and stored at -70ºC until final analysis. The final analyses were performed at a tertiary care ISO (International Organization for Standardization), RIQAS (Randox International Quality Assessment Scheme) and World Federation of Hemophilia’s (WFH) IEQAS (International External Quality Assessment Scheme) certified diagnostic referral center of Pakistan. Samples were transported between the laboratories on dry ice.

First and second line coagulation tests were performed on STA COMPACT MAX® (STAGO, US) hemostasis analyzer. Some of the results were cross checked on SYMEX CA 1500® coagulation analyzer. Patients with FVIII and FIX levels less than 40% were diagnosed as FVIII and FIX deficient, respectively.

**Statistics**

The data was recorded and analysed using IBM SPSS® (version 20) software. Simple arithmetic analyses (mean, standard deviation and percentages) were deduced and statistical tests were applied where feasible. The results were arranged in figures, tables and graphs using SPSS and Edraw Max® (version 6.8).

**RESULTS**

In the initial survey, it was found that only transfusion centers and hemophilia care centers of provincial and federal capitals had registries of FIX deficient patients. Total number of FIX deficient patients registered at these facilities was 83. Only 32 patients could be contacted (the rest either had not provided any contact details to the facility or had their contact numbers changed), of whom 30 patients consentingly volunteered for the study.

Primary disease description in the registries at health care facilities was limited (details provided in Supplementary Document). Severity status could be defined in only 08 (26.66%) patients. Rest of the 22 (73.33%) patients could not be categorized because their factor levels were not formerly investigated. Diagnoses were labeled upon advice of consultant pathologist with laboratory evidence of mixing studies or factor assays. No system of verification of laboratory test reports was in practice.

The study patients ranged in age from 04 years to 44 years. Among the study participants, one patient was female. Geographically, the patients were randomly scattered across the province.

In our study, we found that the registries at transfusion centers and hemophilia care centers did not have any mild HB case in their record (Table I). The registered FIX deficient patients’ pool was found to have a mean age of 17.39±9.8 years. True HB patients were only 18 (60.0%), whereas 09 (30.0%) patients were found to have FVIII deficiency and 3 (10.0%) patients had hypofibrinogenemia. Prophylactic treat-
ment was observed by only 8(26.6%) patients, that too after being affected with arthropathies. It was discovered that none of the patients had regular provision of FIX concentrates and that all the patients were using fresh frozen plasma (FFP) as the primary therapeutic modality.

Diagnoses in our study patients were based on mixing studies and/or factor assays. All the patients (n=8, 100%) previously diagnosed with factor assays were found to have been diagnosed correctly (Figure 1). Among those who were diagnosed previously with mixing studies, only 08 (44.44%) had FIX deficiency while 10 (56.66%) were misdiagnosed. Outcome of these two tests was compared with Fisher’s exact test; the difference turned out significant (P-value 0.0095).

In our study, we found that complete profile for mixing studies, which include mixing the test plasma with normal pooled plasma, adsorbed plasma and aged plasma/serum, was tested in only 03 out of 23 cases (Figure 2). In a few instances (n=04), the previous diagnosis of FIX deficiency was based on circumstan-
DISCUSSION

The overall objective of this study was to estimate the magnitude of diagnostic discrepancy among registered FIX deficient patients of KP province of Pakistan. Our results are affirmative of the fact that labeled HB pool of KP harbors a significant number of FVIII deficient and hypofibrinogenemia patients. Diagnostic discrepancy among FIX deficient patients of Pakistan was anticipated in another study on FVIII deficient patients wherein misdiagnosis was found in 8% of the cases. In the current study, we found that diagnostic validity of factor assays was better than mixing studies. However, studies have shown that mixing studies methods are considerably accurate and specific in diagnosing hemophilia, especially if plasma of FVIII deficient and FIX deficient patients are used instead of aged plasma and adsorbed plasma, respectively. In our case, the flaw seems to be residing with the reagents and/or the test performers. The “high frequency” of discrepancy, in itself, advocates analytical errors to be the main problem rather than pre and post analytical ones. This is in contrast to what is found in studies conducted in well standardized laboratories. Again, the personnel conducting the tests seems to have correctly identified prolongation of aPTT and thus seems less likely to be responsible for the discrepancy. Limitations of aged and adsorbed plasma, on the other hand, are well known and hence more likely cause of misdiagnosis.

KP province comprises approximately 10 million male individuals. Based on global mean prevalence of 1.20 HB patients per 100000 males, an estimated extrapolation in local population turns out to be around 120 (patients). However; the registered FIX deficient patients are 83 in number. Moreover, we found in our study that only 57.6% of these registered patients do actually have FIX deficiency which reduces the count from 83 to 48 patients. These numbers of HB deficient patients in whom factor assays are not performed. Establishment of a total quality management system is the ultimate solution to dealing with laboratory errors. Provision of a reference laboratory is another key solution to analytical discrepancies. Routine standardized evaluation of patients, at least once a year, allows their prospective assessment and enables the physician to predict new or potential problems in their primary stages which guides the treatment plans accordingly. This practice can also effectively sort cases with wrong diagnoses.

The study had the shortcoming of inability to access more than half the registered patients. Exact cause of the discrepant diagnoses could not be found. Another study is needed to experimentally test and prove the possibility of error marked in the study. Clinical outcome should also be figured out in the misdiagnosed cases in comparison to those with correct diagnosis.

Although the sample size is small, frequency of discrepancy found in current study is significant. The findings are especially generalizable to settings where circumstances advocate poor quality control and lack of expertise of laboratory personnel preparing reagents for mixing studies.
In the whole-sum in this study we surveyed a significant proportion (almost 40%) of registered hemophilia B patients of KP, Pakistan. It was found that a large number of these patients were, in fact, not FIX deficient. Most of these misdiagnosed patients were FVIII deficient whereas some had hypofibrinogenemia. It was also found that all the discrepant diagnoses were based on mixing studies. Hence, it is suggested to reconsider diagnosis in all those patients in whom it is based on mixing studies.

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REFERENCES


CONFLICT OF INTEREST

Authors declared no conflict of interest

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Following authors have made substantial contributions to the manuscript as under:

MTHK, AN: Concept & study design, acquisition, analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

AST, TSS, MTHK: Acquisition of data, drafting the manuscript, critical revision, final approval of the version to be published

NF, JA: Drafting the manuscript, final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.