Department of Biochemistry

Newsletter

All India Institute of Medical Sciences,Rajkot

CLINIAL BIOCHEMISTRY & MOLECULAR BIOLOGY BULLETIN

Volume 1 Issue 2 May 2002

Theme of 2022
"Be Aware. Share. Care: Working with the global community as one to improve thalassaemia knowledge."

This issue is dedicated to

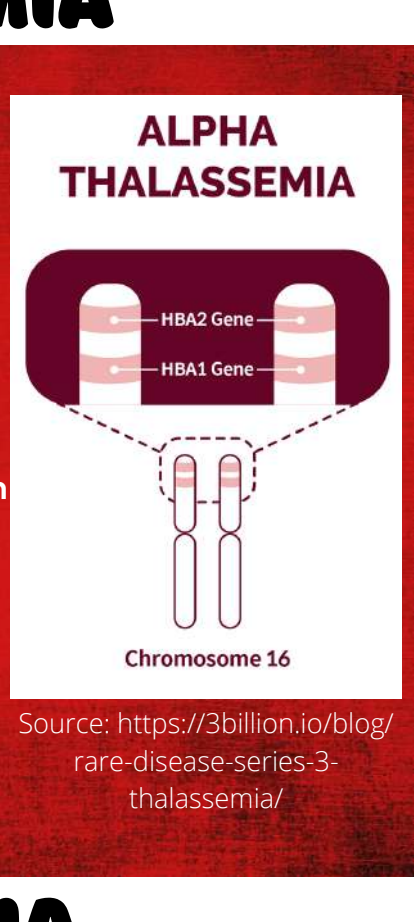
Molecular Genetics of Thalassemia

Thalassemia are inherited blood disorders characterized by decreased hemoglobin production.
Two main types are: Alpha thalassemia and Beta thalassemia.
The severity of the disease depends on how many of the four genes for alpha globin or two genes for beta globin are malfunctioning.
Both α- and β-thalassemia are commonly inherited in an autosomal recessive manner.

α-Thalassemia		β-Thalassemia	
Clinical Classification	Genotype	Clinical Classification	Genotype
Silent Carrier	αα/-α	β-Thalassemia Minor	β^+/β or β^o/β
α-Thalassemia Trait	-α/-α or αα/--	β-Thalassemia Intermedia	β^+/β^+ or β^o/β^+
HbH disease	-α/--	β-Thalassemia Major	β^o/β^o
Hb Bart / Hydrops fetalis	--/--		

ALPHA- THALASSEMIA

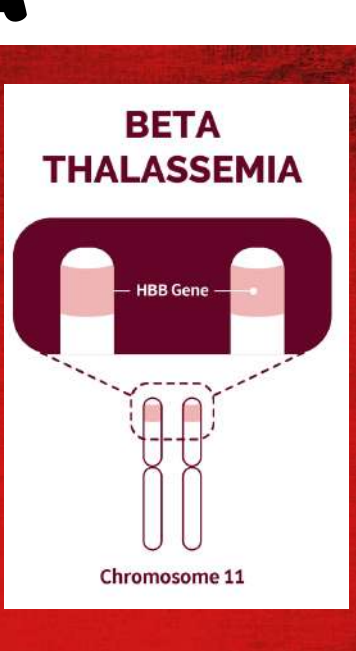
ALPHA GLOBIN GENES ARE LOCATED AT CHROMOSOME 16 (16P 13.3). Mainly 2 types of mutation detected in alpha thalassemia: deletional and non-deletional.Deletional mutations are more common in Alpha-thalassemia. -alpha3.7 and -alpha4.2 are more frequently encountered deletions. Extended deletions, varying from 100 to >250 kb may result in illegitimate recombination, reciprocal translocation, and truncation of chromosome 16. Nondeletion defects are less frequently found in alpha-thalassemia. These defects include single nucleotide substitutions or oligonucleotide deletions/insertions in regions critical for alpha globin gene expression. Several mechanisms such as abnormalities of RNA splicing and of initiation of mRNA translation, nonsense and frameshift mutations, in-frame deletions, etc are seen in alpha-thalassemia.



Source: https://3billion.io/blog/rare-disease-series-3-thalassemia/

BETA- THALASSEMIA

Non-deletion mutations, especially, point mutations commonly seen in β-thalassemia which are single nucleotide substitutions or oligonucleotide insertions/deletions affecting β gene expression. Single base substitutions, small insertions, or deletions within the gene or its immediate flanking sequences are also common.
These mutations affect β-globin expression in 3 different mechanisms: mutations leading to defective β-gene transcription (promoter and 5′ untranslated region [UTR] mutations), mutations affecting messenger RNA (mRNA) processing (splice-junction and consensus sequence mutations, polyadenylation, and other 3′ UTR mutations), and mutations resulting in abnormal mRNA translation (nonsense, frameshift, and initiation codon mutations).
Deletional mutations are less common in β-thalassemia. Deletion of portion of structural gene, enhancer region or promotor region can affect functionality of the beta globin gene significantly.



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Experts Talk

Dr. Pinky Meena
Asst. Prof Pediatrics

Thalassemia is one of the most common preventable single gene disorders. In severe form it is associated with chronic, life-impairing and life-threatening diseases with inherent serious health sequelae that can lead to disability or death. Unfortunately, a large number of children in our country continue to be born and suffer from such disorders mainly due to lack of awareness and lack of a comprehensive programme and systematic strategies to prevent them. Data on the prevalence of silent carriers of thalassemia ranges from 2.9-4.6%. It is estimated that about 10000-15000 babies with Thalassemia Major (TM) are born every year.
The only cure available for these children with thalassemia major is hematopoietic stem cell transplant (HSCT). However, this percolates only to a few patients because of cost, limited BMT centres, or unavailability of a suitable HLA matched donor. Therefore, the mainstay of treatment is a regimen of regular blood transfusions followed by adequately monitored iron chelation therapy.
Considering the magnitude of the problem and the cost implications of management, suitable control measures need to be undertaken urgently. Primary prevention includes identifying the carriers and avoidance of marriage of carrier couples and secondary by preventing the birth of affected child through prenatal diagnosis. In countries like Cyprus, Italy, and Canada where successful screening programme in high schools and young adults for Thalassemia has led to decrease in the incidence of thalassemia.

MOLECULAR BIOLOGY & CLINICAL CHEMISTRY (MCC) UPDATES

THALASSEMIA CAN BE DIAGNOSED BY SEPARATION AND QUANTIFICATION OF HEMOGLOBIN FRACTIONS BY ELECTROPHORESIS OR HPLC.
MOLECULAR DIAGNOSIS OF THALASSEMIA CAN BE DONE WITH PCR AND NEXT GENERATION SEQUENCING TECHNIQUES.
THESE TECHNIQUES CAN SPECIFICALLY IDENTIFY CAUSATIVE MUTATIONS SUCH AS SINGLE NUCLEOTIDE SUBSTITUTIONS, INSERTIONS, SHORT OR LONG DELETIONS. THESE MOLECULAR BIOLOGY TECHNIQUES CAN BE USED FOR PRENATAL DIAGNOSIS OF FOETUS OF THALASSAEMIC COUPLE.

Recently, the Clinical Biochemistry service laboratory has got HPLC analyser installed in AIIMS, Khanderi. Our lab is now equipped to perform thalassemia diagnosis

Tid-Bits From History

Banting & Best: Discovery of Insulin ,July 27, 1921

July 27 marks one of the most important days in diabetes treatment history. On that date in 1921, Dr. Frederick Banting, a Canadian surgeon and Charles Best, a medical student, successfully isolated the hormone insulin for the first time. The breakthrough research took place at the University of Toronto, where Banting and Best successfully isolated insulin from dogs, produced diabetes symptoms in the animals, and then provided insulin injections that produced normal blood glucose levels. Dr. Banting shared his success with Professor John Macleod.

INSULIN TREATMENT BEGINS FOR HUMANS

Plans were quickly underway for an insulin treatment for people. Next, they extracted insulin from the pancreases of cattle from slaughterhouses. On January 11, 1922, 14-year-old Leonard Thompson became the first person to receive an insulin injection as treatment for diabetes. The first caused an allergic reaction. A refined process was quickly developed to improve the cow pancreas from which the insulin was derived, and Thompson's second dosage was successfully delivered twelve days later on January 23. The teenager's condition improved dramatically. Diabetes, which had been regarded as a fatal disease, could finally be managed!

Nobel Prize awarded to Banting and Macleod
By 1923, insulin had become widely available in mass production, and Banting and Macleod were awarded the Nobel Prize in medicine.
Charles Best, being a graduate student, was not included.
Banting recognized Best's involvement by sharing the award money.

Biochemical Basis of Tear-Inducing Property of Onions

Slicing/dicing/Crushing of onion ruptures millions of cells and causes the release off an intracellular component amino acid sulfoxides along with an enzyme Alliin Lyase (EC 4.4.1.4; Allinase)

- Allinase catalyses a biochemical reaction to break amino acid sulfoxides and generates sulfenic acids.
- A specific sulfenic acid namely 1-propenesulfenic acid is rapidly rearranged by another enzyme viz Lachrymatory Factor synthase, giving rise to syn-propanethial-S-oxide, more commonly known as onion lachrymatory factor
- This compound (syn-propanethial-S-oxide) is volatile, even at room temperature. The gas diffuses through the air and on contact with the eye, it stimulates sensory neurons creating a stinging, painful sensation. Lacrimal glands produce tears in response to dilute and flush out the irritant.
- The characteristic odour and special flavour in onion is also due to these volatile chemicals.

Laboratory Touchup

Laboratory Errors and its Evaluation using Quality Indicators

In the inaugural issue of Clinical Biochemistry and Molecular Biology bulletin {2022;April Vol 1 (issue 1)}, 25 Preanalytical Quality indicators (QI) were described under the title "laboratory errors and its evaluation using Quality Indicators. In continuation, enduring quality indicators specified for intra-analytical phase (n: 06; Table 1), postanalytical phase (n: 12; Table 2), support processes {(n: 05; Table 3) two for staff competence, two users' satisfaction, one efficiency of laboratory information system} and outcome measures (n: 05; Table 4) are labelled herewith in consort with its reporting systems, mode of data collection and its relevant code with priority.

INTRA-ANALYTICAL PHASE ERRORS AND QIs

Analytical phase commences with the specimen under analysis is prepared for testing, and it ends when the test result is interpreted and verified in the laboratory. Improper processing of a specimen, interfering substances with assay performance can distress test results in this phase [1]. Establishing and verifying test method performance specifications as to test accuracy, precision, sensitivity, specificity, and linearity are other areas where errors can occur in the analytical phase [2]. A significant decrease in error rates in the analytical phase (7-13%) compared to pre (46 to 68%) and post analytical phases (19-47%) is due to advancement in automation; standardization and optimization of reagents, analytical techniques, improved training of the laboratory staff, advances in information technology and above all by implementing internal quality controls (IQC) and external quality assessment (EQA) [3-4].

POST-ANALYTICAL PHASE ERRORS AND QIs

it is the concluding phase of laboratory work in which laboratory results are appraised and it involves different personnel as per competencies [5]. The post-analytical phase can be further divided into a phase inside the laboratory and a phase outside the laboratory. Inside the laboratory phase errors includes erroneous validation of analytical data, failure in reporting/addressing the report, excessive turn-around-time, improper data entry and manual transcription error, failure/delay in reporting critical values. Whereas, major errors in phase outside the laboratory includes delayed/missed reaction to laboratory reporting, incorrect interpretation, inappropriate/inadequate follow-up plan, failure to order appropriate consultation.

- To facilitate the introduction into practice for each indicator, an order of priority has been assigned based on the importance of the specific indicator and the difficulty of data collection (one the highest priority, four the lowest). The QIs with priority one, which are mandatory, are to be put into practice first (order of priority: 1 = mandatory; 2 = important; 3 = suggested; 4 = valued) [1].

TABLE 1: QUALITY INDICATORS FOR KEY PROCESSES – INTRA-ANALYTICAL [1]

Quality Indicator	Reporting Systems	Data Collection	Code
PRIORITY - 1			
Test uncovered by an IQC	**Percentage of:** Number of tests without IQC/Total number of tests in the menu.	a) count number of tests without IQC b) count total number of tests in the menu c) calculate percentage	Intra-IQC
Unacceptable performances in IQC	**Percentage of:** Number of IQC results outside defined limits/Total number of IQC results	a) count number of IQC results outside defined limits b) count total number of IQC results c) calculate percentage	Intra-UnIQC
Test uncovered by an EQA-PT control	**Percentage of:** Number of tests without EQA- PT control/Total number of tests in the menu.	a) count number of tests without EQA-PT control b) count total number of tests in the laboratory menu c) calculate percentage	Intra-EQA
Unacceptable performances in EQA-PT schemes	**Percentage of:** Number of unacceptable performances in EQAS-PT Schemes, per year/Total number of performances in EQA Schemes, per year.	a) count number of unacceptable performances in EQA Schemes b) count total number of performances in EQA Schemes c) calculate percentage	Intra-Unac
Data transcription errors	**Percentage of:** Number of incorrect results for erroneous manual transcription/Total number of results that need manual transcription.	a) count incorrect results for erroneous manual transcription b) count results that need manual transcription c) calculate the percentage	Intra-ErrTran
	Percentage of: Number of incorrect results for information system problems/Total number of results.	a) count incorrect results for information system problems b) count total number of results c) calculate the percentage	Intra-FailLIS

TABLE 2: QUALITY INDICATORS FOR KEY PROCESSES – POST-ANALYTICAL [1]

Quality Indicator	Reporting Systems	Data Collection	Code
PRIORITY - 1			
Inappropriate turnaround times	**Percentage of:** Number of reports delivered outside the specified time/Total number of reports.	a) count reports delivered outside specified time b) count total number of reports c) calculate the percentage	Post-OutTime
	Turnaround time (minutes), from sample reception in laboratory to release of result, of Potassium (K) at 90th percentile (STAT).	a) estimate all TAT (minutes), from sample reception in laboratory to release of result, of Potassium (STAT) released in the month b) estimate the 90th percentile	Post-PotTAT
	Turnaround time (minutes), from sample reception in laboratory to release of result, of International Normalized Ratio (INR) value at 90th percentile (STAT).	a) estimate all TAT (minutes), from sample reception in laboratory to release of result, of International Normalized Ratio (INR) (STAT) released in the month b) estimate the 90th percentile	Post-INRTAT
	Turnaround time (minutes), from sample reception in laboratory to release of result, of White Blood Cell (WBC) count at 90th percentile (STAT).	a) estimate all TAT (minutes)), from sample reception in laboratory to release of result, of White Blood Cell (WBC) count (STAT) released in the month b) estimate the 90th percentile	Post-WBCTAT
	Turnaround time (minutes), from sample reception in laboratory to release of result, of Cardiac Troponin (TnI or TnT) at 90th percentile (STAT).	a) estimate all TAT (minutes)), from sample reception in laboratory to release of result, of Cardiac Troponin I (TnI or TnT) (STAT) released in the month b) estimate the 90th percentile	Post-TnTAT
	Percentage of: Number of Potassium results (STAT) released after 1 hour/Total number of Potassium results (STAT)	a) count number of Potassium results (STAT) released after 1 hour b) count total number of Potassium results (STAT) c) calculate the percentage	Post-TATPotH
Incorrect laboratory reports	**Percentage of:** Number of rectified reports by laboratory after the release/Total number of released reports.	a) count number of rectified reports after the release b) count total number of released reports c) calculate the percentage	Post-RectRep
Notification of critical results	**Percentage of:** Number of critical results of inside patients notified after a consensually agreed time (from result validation to result communication to the clinical ward)/Total number of critical results of inside patients to communicate.	a) count critical results of inside patients notified after a consensually agreed time (from result validation to result communication to the clinical ward) b) count total number of critical results of inside patients to communicate c) calculate percentage	Post-InsCR
	Percentage of: Number of critical results of offside patients notified after a consensually agreed time (from result validation to result communication to the general practitioner)/Total number of critical results of offside patients to communicate.	a) count critical results of offside patients notified after a consensually agreed time (from result validation to result communication to the general practitioner) b) count total number of critical results of offside patients to communicate c) calculate percentage	Post-OffCR
PRIORITY - 4			
Notification of critical results (TAT)	Median value of time (from result validation to result communication to the clinical ward) to communicate critical results of inside patients (minutes)	a) estimate the time (minutes) to communicate critical results of inside patients b) calculate the median value of estimated times	Post-InsCRT
	Median value of time (from result validation to result communication to the general practitioner) to communicate critical results of offside patients (minutes)	a) estimate the time (minutes) to communicate critical results of offside patients b) calculate the median value of estimated times	Post-OffCRT
Interpretative comments	Percentage of: Number of reports with interpretative comments impacting positively on patient's outcome/Total number of reports with interpretative comments	a) select a test, or group of tests, that often requires a comment for the correct interpretation of the result b) select a clinical ward and contact a physician in order to analyse together the reports with interpretative comments c) evaluate the clinical actions undertaken on the basis of interpretative comments d) evaluate the patients outcome e) count the positive outcomes f) count the total number of reports with interpretative comments g) calculate the percentage	Post-Comm

TABLE 3: QUALITY INDICATORS FOR SUPPORT PROCESSES [1]

Quality Indicator	Reporting Systems	Data Collection	Code
PRIORITY - 2			
Employee competence	Number of training events organized for all staff, per year	a) count number of training events organizedfor all staff	Supp-Train
	Percentage of: Number of employees that obtained all credits required in a year/Total number of employees	a) count number of credits obtained byemployee b) count total number of credits to be obtained c) calculate percentage	Supp-CME
Client relationships	**Percentage of:** Sum of point given in the enquiry tothe question of global satisfaction of the physician/ Multiplication of the maximum point defined in the enquiries by the number of enquiries	a) sum point given in the enquiry to thequestion b) multiply the maximum point defined in theenquiries by the number of enquiries c) calculate percentage	Supp-Phys
	Percentage of: Sum of point given in the enquiry tothe question of global satisfaction of the patient/Multiplication of the maximum point defined in theenquiries by the number of enquiries	a) sum point given in the enquiry to thequestion b) multiply the maximum point defined in theenquiries by the number of enquiries c) calculate percentage	Supp-Pat
PRIORITY - 3			
Efficiency of Laboratory Information System	Number of Laboratory Information Systemunplanned downtime episodes, per year	a) count number of Laboratory InformationSystem downtime episodes	Supp-FailLIS

TABLE 4: QUALITY INDICATORS FOR OUTCOME MEASURES [1]

Quality Indicator	Reporting Systems	Data Collection	Code
PRIORITY - 1			
Sample recollection	**Percentage of:** Number of patients with recollectedsamples for errors due to laboratory staff/Total number of patients	a) count number of patients with recollected samples for errors due tolaboratory staff b) count total number of patients c) calculate percentage	Out-RecLab
	Percentage of: Number of patients with recollectedsamples for errors not due to the laboratory staff/Total number of patients	a) count number of patients with recollected samples for errors notdue to laboratory staff b) count total number of patients c) calculate percentage	Out-RecOff
Amended results	**Percentage of:** Number of amended results/Total number of released results	a) count number of amended resultsreleased b) count total number of resultsreleased c) calculate percentage	Out-InacR
Safety	Number of incident/adverse events occurred in laboratory concerning the health and safety of laboratory staff	a) count number of incident/adverseevents occurred in laboratory concerning the health and safety of laboratory staff	Out-Adv
	Number of needlestick injury/Total number of venipunctures	a) count number of occurredneedlestick injury b) count total number ofvenipunctures c) calculate percentage	Out-Inj

CONCLUSION

Laboratory testing/services have a significant role in the provision of health care considering its impact on clinical outcomes. In consequence, it is essential to have a knowledge of error rates in clinical laboratories as it enables to evaluate the performance and take corrective measures. This can be achieved by the use of Quality Indicators across the phases of TTP, as well as it serves as a fundamental tool to assess the quality of laboratory services. Thus, Quality indicator data/system should be part of a coherent and coordinated quality improvement strategy, should be constantly collected and reviewed over time to monitor errors and improve performance and patient safety.

References

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"The ability to observe without evaluating is the highest form of intelligence."