CHAPTER 30

MEDICAL SCIENCES
PADIATRICS

Doctoral Theses

01. JINDAL (Ankur)
Prenatal Screening Initiative by Dried Blood Spot: Role for Triaging and Resource Utilization.
Supervisors: Dr Seema Kapoorv and Sudha Prasad
Th 26195

Abstract

Objective: The objective of the study to standardize dried blood spot (DBS) assays against serum in the first trimester. Evaluate the performance of contingent screening, utility, yield, and cost-effectiveness of using QF-PCR in the amniotic fluid in the rapid diagnosis of fetal aneuploidies in the Indian context Materials & Methods- In this study a total of 2150 pregnant women who attended the antenatal clinic of Obstetrics and Gynecology in the Lok Nayak Hospital, New Delhi, were screened for First trimester screening. Dried blood spot as well as blood were collected by venipuncture and finger prick for dual assay and the concentrations were measured by Auto DELFIA R (Perkin Elmer, Turku, Finland). In final risk between 1:250 to 1:1000, NT value were incorporated and again final risk were calculated with a final cut-off 1:250. All high risk women underwent for amniocentesis/Chronic villus sampling and QF-PCR was performed on these samples for rapid diagnosis of fetal aneuploidies. Results- DBS assay was performed in 2150 cases for β- hCG and PAPP-A. The two methods showed agreement in 91.9% of the cases and disagreement in 8.1% of the cases. Out of 2150 cases 486 (22.6%) showed the intermediate risk. NT was done and incorporated, only 40 women have high risk > 1:250 were categorized in to high risk and 53 women have risk 1:250 to 1:1000 were underwent for second trimester screening. NT measurement were required only in 22.6% women. Out of 40 only 24 women opted for invasive testing and amniocentesis were performed. QF-PCR were performed to all the women in which 4 women showed the high risk for trisomy 21. Conclusion- The high concordance between serum and DBS fulfilling the objective of implementing the dried blood spots as resource to do vital studies. By the use of this dried blood spot technique, prenatal screening can be started as a nationalized program in future.

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02. LOMASH (Avinash)
Study the Additive Predictability of NON-HLA Markers in the First – Degree Relatives of Children with Celiac Disease.
Supervisors: Dr.Seema Kapoor, Dr.Vineet Vijay Batra, Dr. A.S.Puri, Dr.Praveen Kumar and Dr. A.P.Dubey
Th 26193

Abstract

Celiac disease (CD) is a chronic inflammatory, multiorgan autoimmune disease that affects the small bowel preferentially in genetically predisposed individuals, precipitated by gluten present in cereals such as wheat, barley and rye (Di Sabatino and Corazza 2009). It is an immune-mediated systemic disorder elicited by the gluten and related prolamines (gliadin in wheat, hordein in barley, secalin in rye and avenin in oats) and is characterized by the presence of a different combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA DQ2 and DQ8 haplotypes and enteropathy (Husby et al. 2012a). The initial nomenclature for CD was coeliac sprue, gluten-sensitive enteropathy, or non-tropical sprue (Al-Toma et al. 2019). The first description of the CD was given in second century AD by a Greek physician (Paveley 1988). The first mention of CD in India was in 1966 by Prof BNS Walia and Prof RC Mishra in pediatric and adult patients, respectively (Misra et al. 1966; Walia et al. 1966) suggesting the differences between different population subgroups. The factors suggested for this change include both environmental and hygiene related factors, quality and quantity of gluten ingested and the changing infant feeding pattern (Catassi et al. 2014). The possible reasons for the increasing prevalence of CD may also be attributed to the easy availability of diagnostic tests and screening of the high-risk population, i.e. first-degree relatives. It is yet unclear whether, the prevalence of CD is truly increasing or there isperceived increase, because of the increased awareness and active case-finding among the high-risk susceptible groups.

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03. SOMESH KUMAR

Clinical, Biochemical and Selected Mutational Spectrum of Mitochondrial and Peroxisomal Disorders in Indian Patients.

Supervisors: Dr. Seema Kapoor Dr. Ravindra Kumar Saran Dr. Madhulika Kabra

Th 26194

Abstract

Mitochondrial and peroxisomal disorders are the two common yet complex neurometabolic disorders. The spectrum of these two sets of disorders would help in understanding the pathophysiology and also aid in developing the possible therapeutic targets, which may result in better patient management. The primary aim of this study was to evaluate diagnostic utility of Morava et al's diagnostic criteria for mitochondrial disorders, and Krause et al's diagnostic criteria for peroxisomal disorders. A total of 86 patients [46 mitochondrial disorders (MD) + 40 peroxisomal disorders(PD)] were recruited. A number of biochemical tests (Liquid or gas chromatography mass spectrometry) , and molecular tests including single gene sequencing, mitochondrial genome sequencing, targeted gene sequencing, whole exome sequencing (wherever required), radiology assays (Loes score in adrenoleukodystrophy patients, and oxidative stress assay (such as mitochondrial membrane potential, reactive oxygen species, damages (single-double strand both) were carried to evaluate the diagnostic utility of Morava et al's and Krause et al's diagnostic criteria respectively. Out of 46 patients, 36 patients belonged to the primary mitochondrial disorder group, which was further categorized under syndromic (N=14) and non-syndromic (N=22), rest 10 patients were grouped in

secondary mitochondrial disorders. Another 40 patients recruited under peroxisomal disorders, categorized as 6 with childhood cerebral adrenoleukodystrophy, 13 with adrenoleukodystrophy, 9 with adrenomyeloneuropathy, 1 with Addison like phenotype and 6 Asymptomatic and remaining 5 patients' from peroxisomal biogenesis defect (Zellweger Spectrum disorders subgroup) FGF 21, GDF 15 , Reactive oxygen species, DNA damages ($p<0.001$) were significantly elevated in patients MD's, whereas C26:0 Lyso PC, DNA damages ($p<0.001$) were significantly elevated in patients PD's, CONCLUSION Inclusion of various other biomarkers such as DNA damages assay, reactive oxygen species, mitochondrial membrane potential, antioxidant capacity, along with biochemical and radiological findings results in the increase in sensitivity of these two diagnostic criteria.

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04. VARUGHESE (Bijo)

Flow injection Analysis Method (FIA) for the Second- Tier Estimation of Methylmalonic Acid (MMA), Methylcitric Acid (MCA), Homocysteine (HCY) and Succinylacetone (SUAC) on Residual Dried Blood Spot from the Primary Newborn Screening.

Supervisors: Dr. Seema Kapoor, Dr. Siddarth Ramji and Dr. Alpana Saxena

Th 26196

Abstract

This study was conducted to develop a flow injection analysis method for the secondtier estimation of methylmalonic acid, methylcitric acid, homocysteine and succinylacetone from residual dried blood spot samplesobtained from primary newborn screening. The primary markers for these disorders in primary newborn screening lack the required specificity and sensitivity, and often provide inconclusive results because of their non-specific elevation due to multiple causes. This study was undertaken to provide a differential diagnosis for disorders like methylmalonic aciduria, propionic acidemia, homocystinuria and tyrosinemia type 1 using the same residual dried blood spots, thereby reducing the additional recall, re-sampling and retesting. A total of 150261 newborn samples were included in our study from the newborn screening initiative in Delhi state funded by the Department of Science and Technology. All neonates were enrolled as per our inclusion criteria. Residual newborn dried blood spot samples belonged to neonates whose initial analyte levels for propionyl carnitinine (C3), methionine (Met) and tyrosine (Tyr) were beyond the kit cut-off values i.e. C3 >8.2 µmol/L, Met >52 µmol/L and Tyr >327 µmol/L. First, an in-house analytical method was developed in aqueous controls and calibrator by spiking the known concentration of deuterated standards for methylmalonic acid, methylcitric acid, homocysteine and succinylacetone in the suitable aqueous solution suitable for each analyte. The various compound and ion source related parameters were optimized by direct infusion of the standards using a syringe pump method. The optimized parameters were later used for the samples spiked with known concentration of standard in blood and spotted onto dried blood spots. The extraction solution and methodology for sample preparation was optimized. Secondly, the developed methodology was validated using the clinical laboratory standard institute (CLSI) guidelines. Once validated, the method was followed for routine samples which required a second tier testing.

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