# Clinical Indications and Outcome of Biotinidase Deficiency Screening among Children and Youths in a Scottish NHS Region Between 2014 and 2016

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## Abstract:

Biotinidase deficiency (BTD) is an autosomal recessive metabolic disorder characterized by neurodevelopmental and cutaneous disorders. Individuals with BTD have either homozygous or compound heterozygous variants of biotinidase (BT) enzyme. We aimed to analyse the pattern and outcome of investigations for BTD among children and young people in a Scottish NHS Board. We retrospectively analysed the clinical and laboratory data of all children within the Fife area who were screened for BTD between July 2014 and July 2016. BTA levels ranged between 2.7 and 14.1 nmol/min/mL from a total of 191 patients. 262 tests were requested for 243 children aged between 1 month and 17 years-6 months (Mean 70 months). 75 of the samples (29%) were ordered to be repeated. 59 samples from 53 patients (22%) could not be analysed for reasons including "insufficient sample" (34), "unsuitable bottle" (10), "missed in error" (7), and "samples leaked in transit" (3). The commonest indications for BTD screening were developmental delay (63%) and social communication concerns (49%). The majority (93%) of the tests were requested by either the Consultant or Specialist Community Child Health (CCH) Paediatricians. None of the 191 patients analysed had BTD. However a substantial proportion of the patients (22%) could not be analysed due to various problems with their blood samples. This study has practical implications for routine clinical care and investigation for children and young people with Developmental or Learning Disabilities. A larger prospective study is required to verify the true prevalence of BTD in the population.

## 1. Introduction

Biotinidase deficiency (BTD) is an autosomal recessive metabolic disorder characterized by neurodevelopmental and cutaneous disorders. Individuals with a biotinidase deficiency have either homozygous or compound heterozygous variants of biotinidase (BT) enzyme. BT is an amidohydrolase enzyme which plays a critical role in releasing free biotin from dietary biotin (biocytin and biotinylated peptides) prior to absorption. One hundred and sixty-two genetic variants of *BTD* that alter biotinidase activity have been reported so far. Biotinidase activity (BTA) measurement may also be artifactually low due to enzyme lability, premature birth, and jaundice [1].

Biotin-dependent carboxylases catalyze the fixation of bicarbonate in organic acids and play crucial roles in the metabolism of fatty acids, amino acids and glucose. Biotin is also covalently attached to histones; biotinylated histones are enriched in repeat regions in the human genome and appear to play a role in transcriptional repression of genes and genome stability [2,3]. In 2004, the incidence of biotinidase deficiency in Europe was estimated to be 1:47 486, according to statistics data collected through newborn screening programmes in seven countries (Austria, Belgium, Germany, Italy, Spain, Sweden, and Switzerland) [4].

Symptoms of BT deficiency include seizures, hypotonia, ataxia, dermatitis, hair loss, respiratory problems, developmental delay, hearing and vision problems, intellectual (learning) disability, ketolactic acidosis, organic aciduria and also foetal malformations [3]. All symptomatic individuals improve with oral pharmacological doses of biotin.

BTA can be determined by colourimetric or fluorimetric enzymatic assays in serum or plasma. Individuals with profound BTD have enzyme activity less than 10% of mean normal serum BT activity and those with partial deficiency have enzyme activity between 10 and 30% of mean normal serum activity. Very few patients with residual BT activities >1% but in the profound deficiency range, may however remain asymptomatic even without treatment. Children with partial deficiency often remain largely asymptomatic but may eventually develop symptoms typical of profound BTD, particularly when they are stressed by an infectious disease or moderate gastroenteritis. It is now recommended that all children with partial deficiency should be treated with oral biotin in order to avoid the possibility that they would develop symptoms [1,5].

Suspected Developmental Delay (SDD) and Learning disability (LD) are common indications for BTD screening in children and young people. SDD is highly prevalent in infancy and preschool age and constitutes between 5% and 16% of the general paediatric population worldwide [6,7]. Developmental delay is often diagnosed when there is a delay in areas of speech, language, cognitive, fine- and gross-motor, and social adaptation skills. Global Developmental Delay (GDD) is defined as a significant delay in two or more domains of development. The aetiology of GDD is heterogeneous, and includes prenatal, perinatal, postnatal, or undetermined causes. This makes its diagnosis to be very difficult; and up to 62% of children with GDD may have undetermined aetiology [8]. LD is a lifelong, debilitating condition affecting 2% to 3% of population in many advanced countries [9]. It is defined by deficits in cognitive functioning (IQ <70) and adaptive skills, diagnosed before the age of 18 years. LD is often termed as GDD in children below 5 years of age. Associated comorbidity in LD/GDD is significant and includes Epilepsy, Autism, psychiatric/behavioural disturbances, sensory deficiencies and systemic organ involvement (eg, congenital heart defects, liver disease).

LD is associated with the highest health care costs of any disease, nearly equalling the combined economic impact of stroke, heart disease and cancer [10]. Given the aetiological heterogeneity of LD/GDD, including acquired environmental factors such as infections, trauma and toxic agents, complexly interacting with genetic factors, diagnostic evaluation of children with LD/GDD is a challenge for Neurologists, Geneticists and Paediatricians. Limited evidence has estimated that the prevalence of BTD is higher than in the general population among children with GDD/LD, especially if there are associated brain abnormalities on MRI scans [11,12]. In the absence of robust research evidence, several clinicians have conflicting views about the usefulness or otherwise of screening children with GDD/LD or unexplained neurological symptoms for BTD [13,14,15].

The literature suggests low diagnostic yields associated with metabolic testing (0.8% to 2.5%) of children with GDD/LD, but investigation of inborn errors of metabolism (IEM) in these children is regarded as highly cost-effective because it represents the largest category of genetic conditions amenable to causal therapy [9]. This low yield has been partially attributed to the recent advent of tandem mass spectrometry, which has further increased the yield of universal Neonatal Screening programmes for metabolic disorders and has decreased the number of children who present with undiagnosed GDD later in life [16].

Several strategies to improve the yield of metabolic testing among children with GDD/LD have been recommended. A pre-selection of cases for detailed metabolic screening using a stepwise or checklist approach (including the presence of dysmorphology, hepato-splenomegaly, and ophthalmologic and neurologic findings etc) has been suggested, which could increase the yield to 13.6% [17]. Other authors have recently recommended a two-tiered diagnostic protocol prioritizing investigation for treatable genetic conditions presenting with GDD/LD. The first tier level which involves biochemical tests that potentially identify 60% of the currently known treatable inborn metabolic diseases (IMD), are offered to all patients with unexplained GDD/LD. This includes Plasma acylcarnitine profile, amino acids, homocysteine, copper, ceruloplasmin, ammonia and lactate and Urinary creatine metabolites, glycosaminoglycans, oligosaccharides, organic acids, pyrimidines and purines. The second tier levels includes more specific tests required for the remaining 40% of the treatable IMDs, such as primary molecular analysis, and are reserved for fewer selected cases with high index of clinical suspicion [18].

Some evidence-based guidelines have recommended BTD screening as first line investigation for children with moderate or severe LD/GDD [19]. Other authors have suggested a strictly clinical-based model of screening for BTD only in patients with relevant clinical presentation [20]. Several authors have advocated for universal newborn screening of BT activity, rather than clinical-based screening [5]. Their arguments include the wide availability of both inexpensive and effective screening and treatment to avert early developmental disorders and life-long neurological abnormalities. Furthermore, many individuals with BTD may remain asymptomatic for a long time and may not present with classical symptoms that will alert clinicians to screen and treat them early [21,22,23,24].

In the United Kingdom, BTD has been considered several times and most recently in July 2013 by the National Screening Committee. The Committee has not recommended that it should be added to their Newborn Screening programme. This

recommendation is however currently being reviewed and public consultation period has been announced in October 2017, initially for three months [25].

## 2. Materials and Methods

### 2.1. Clinical and Laboratory Analysis

We aimed to analyze the pattern and outcome of investigations for BTD among children and young people in the Scottish Fife NHS Regional Area. We retrospectively reviewed the clinical and laboratory data of all children throughout NHS Fife area screened for Biotinidase activity (BTA) over a two- year period between July 2014 and July 2016. Standardized demographic and referral information and the range of clinical presentation were collected for each patient.

Biotinidase activity was measured in plasma using a colourimetric assay, where free biotin and p-aminobenzoic acid (p-ABA) are released from cleavage of biotin-4-amidobenzoic acid (biotin-p-ABA). The p-ABA is then diazotised and coupled to a napthol reagent resulting in the formation of a purple product which is measured by Spectro-photometric methods.

Profound biotinidase deficiency is defined as levels <0.7 nmol/min/mL and partial deficiency between 0.7 and 2.1 nmol/min/mL. Newborn infants in the partial deficient range should have repeat samples taken when they are $3 - 6$ months of age to determine their status.

### 2.2. Distribution of Socioeconomic Deprivation

Using the residential postcodes, we identified the Data Zone for each patient. The socio-economic status of each child was determined using the latest Scottish Index of Multiple Deprivation (SIMD) 2016 published in August 2016. It is a relative measure of deprivation across 6,976 data zones in Scotland, with roughly equal population of approximately 760. It assigns a ranking from 1 (corresponding to the most deprived area) to 6,976 (the least deprived area) in Scotland. All ranked 6976 data zones are grouped into 5 bands (quintiles), each containing 20% of the data zones, or into 10 bands (deciles), each containing 10% of the data zones. Quintile 1 and Decile 1 contains the 20% and 10% most deprived data zones respectively [26].

### 2.3. Statistical Analysis

Spearman's rank correlation coefficient (http://www.socscistatistics.com/tests/spearman/default2.aspx) was used to determine the relationship between different age groups. Other descriptive statistics used was chi square (with Yates correction when relevant) for comparison of proportions among groups of patients (http://www.socscistatistics.com/tests/chisquare2/Default2.aspx) and t-test for comparison of two means (https://www.medcalc.org/calc/comparison_of_means.php). Analysis of variance (ANOVA) was used when testing for differences between three or more means (http://statpages.info/anova1sm.html). Statistical significance is accepted at the p value of <0.05.

## 3. Results and Discussion

### 3.1. Local Services Description

NHS Fife is one of the fourteen regional boards of NHS Scotland. Its services consist of two main hospitals supported by a network of Community and Day Hospitals. The Primary care services such as GPs and pharmacies are contracted through the three Community Health Partnerships (CHPs) within the local Authority. NHS Fife contains six Local Management Groups and seven Health & Social Care Partnership Localities – Levenmouth, Glenrothes, NE Fife, Cowdenbeath, Dunfirmline, Kirkcaldy and South West (SW) Fife.

Fife is the third largest of the 32 local authority areas in Scotland, consisting of a large rural area with a total estimated population of 370,330 (6.9% of Scotland's population), including over 64,305 children aged 0-15 years (17.4%), covering a more densely populated area of 1325 sq km (1.7% of Area of Scotland). It is bounded to the north by the River Tay and to the south by the River Forth. There were 3,889 live births to Fife residents during 2014 [27]. 36% of its 174,427 households live in fuel poverty, with £340 median weekly income (after housing) [28].

The local Paediatricians in NHS Fife generally followed the evidence-based guidelines from McDonald et al [19] published in 2006, offering BTD screening as first line investigation for childhood GDD/LD. A directive to all clinicians from October 2016 advised the implementation of the National Metabolic Biochemistry Network [20] guidelines for BTD, which offered screening to only clinically suspected cases, as second-line tests, mainly due to insufficient laboratory yield and financial prudence.

### 3.2. Epidemiology

A total of 262 tests (equivalent to 2 tests per 1000 child population per year) were requested for 243 children aged between 1 month and 17 years and 6 months (Mean 70 months, SD 42 months, Median 60 months). This is equivalent to 3.8 per 1000 children population. Male (181): Female (61) ratio was 3:1. Males were on average 15 months younger than females when they were tested (66 vs 81 months) (t-test -2.47, p value 0.014).

The largest age group was represented by 0 to 4 years (50%) and there were only three patients (1%) in the 16-19 years group (Table 1).

*Table 1. Showing the age distribution of the patients*

| Age Band | No_Pts | Percent (n=243) | Avg_BTA levels | Rejected Samples (%) | Percent (n=53) |
|---|---|---|---|---|---|
| 0-4 | 121 | 49.8 | 9.1 | 23(19%) | 43.4 |
| 5-9 | 92 | 37.9 | 8.5 | 20(22%) | 37.8 |
| 10-15 | 27 | 11.1 | 9.1 | 9(33%) | 17 |
| 16-19 | 3 | 1.2 | 4.7 | 1(33%) | 1.9 |
| **Total** | **243** | | | **53** | |
| rho[&] | -1 | -1 | -0.63 | 0.95 | -1 |
| P value | <0.001* | <0.001* | 0.37 | 0.051 | <0.001* |

Legend:

[&] Spearman Rank Correlation

### 3.3. Seasonal Variations

The number of patients referred for BTD screening increased progressively between late 2014 and early 2016 (Table 2). There was no significant difference between the age and gender ratio of the patients throughout the study period.

***Table 2.*** *Showing the seasonal variation among the children screened for BTD*

| Time Period | No_Pts | Avg_Age | Avg_Diag | M:F Ratio |
|---|---|---|---|---|
| **2nd Half 2014** | 44 | 61 ±37.6 | 3 ±1.3 | 7.8:1 |
| **1st Half 2015** | 66 | 71 ±38.2 | 2.9 ±1.7 | 2.1:1 |
| **2nd Half 2015** | 61 | 67 ±43.7 | 2.7 ±1.6 | 3:1 |
| **1st Half 2016** | 72 | 77 ±44.3 | 2.6 ±1.4 | 2.4:1 |
| F score/ Chi sq[&] | | 1.5 | 0.84 | 6.54[&] |
| P value | | 0.22 | 0.48 | 0.09 |

Legend:
Avg_Age: Average age (in months)
Avg_Diag: Average number of diagnosed problems

### 3.4. Sample Rejection Rates

75 samples from 71 patients were ordered to be repeated. 16 patients had their samples repeated once and two patients had their samples tested twice, giving a repeat sampling rate of 19/75 (25%). 59 samples from 53 patients (22%) could not be analysed for reasons including "insufficient sample" (34), "unsuitable bottle" (10), "missed in error" (7), and "samples leaked in transit" (3). Table 3 highlights the reasons for sample rejections and repeat testing rates. The largest number of rejected samples was among the youngest patients (43%) but the largest proportion of rejected samples was among the oldest children who were 10 to 17 years old, where every one in three samples were not processed (Table 1).

***Table 3.*** *Showing various reason for rejection of the blood samples*

| Reasons for rejection | All samples | Percent (n=75) |
|---|---|---|
| Insufficient | 41 | 54.7 |
| Unsuitable, Heparin specimen required | 13 | 17.3 |
| Test missed in error | 6 | 8 |
| Leaked in transit | 3 | 4 |
| Low Values | 2 | 2.7 |
| No specimen received | 1 | 1.3 |
| Mislabelled | 2 | 2.7 |
| Test not indicated | 2 | 2.7 |
| Unsuitable | 1 | 1.3 |
| Regret request missed | 1 | 1.3 |
| No Reasons | 3 | 4 |
| **Total** | **75** | |

### 3.5. Laboratory Outcome of BTD Screening

Results of BTA were analysed for 191 patients with age ranging between 1 month and 17 years 6 months (Mean 68 months, SD 39.5 months, Median 59 months). BTA levels ranged between 2.7 and 14.1 nmol/min/mL (mean 8.9, SD 2.2, Median 9.1). 5 patients had BTA values below the reference value of 4. One of them on repeat increased from 2.7 to 3, and another patient's value increased from 2.7 to 8.7 within two months. The remaining 3 patients had BTA values between 3 and 4 which were not repeated, and were not considered clinically to have BTD. They included an 8 yr

old girl presenting with GDD, ADHD and sleep problems; 14 yrs old boy with emotional problems and a 21 month old boy with social communication concerns. None of the 191 patients were deemed to have BT deficiency both clinically and biochemically.

### 3.6. Clinical Indications for BTD Screening

Each patient had an average of 2.6 diagnosed clinical conditions, ranging from one to seven. These clinical indications were grouped into eight categories: developmental delay, social communication, neurological symptoms, congenital defects, genetic disorders, dysmorphism, emotional and behavioural disorders (EBD) and other general paediatric specialties (endocrine, respiratory or orthopaedic) (Table 4). Most of the patients belonged to two or more diagnostic categories.

The commonest indication for the BTD screening among the patients was Developmental Delay (63%).  Developmental delays presented mainly as GDD (85 patients), LD (55 patients), Speech/Language delay (SALD) in 34 patients and Developmental Coordination Disorder (DCD) (18 patients). 120 patients (49%) had social communication concerns and were either under suspected or already diagnosed with Autism Spectrum Disorder (ASD) (61 patients). EBD presented as Sleep difficulties (52 patients), behaviour problems including Oppositional Defiant Disorder (ODD) (46 patients), Attention Deficit and Hyperactivity Disorder (ADHD) (33 patients), Emotional problems (8 patients) and Foetal Alcohol Spectrum Disorder (FASD) (6 patients).

53 patients (22%) had neurological symptoms such as sensory (14 patients), visual (including nystagmus) impairments (14 patients), Epilepsy (10 patients), hypotonia (10 patients), hearing impairments (3 patients), infantile spasm, Microcephaly, mMacrocephaly, hypertonia, Dystonia, unsteady gait, gray matter heterotopias, Periventricular Leucomalacia (PVL), Ohtahara Syndrome and Cerebral Palsy.

Approximately two thirds of the patients (149) had only Developmental Delay (DD, 78 patients) (32%) or only Social Communication concerns (SCC, 71 patients) (29%) as their only presentations.  46 other patients (19%) presented with a combination of both DD and SCC, while 30 other patients (12%) presented with DD combined with other co-morbidities including neurological symptoms, dysmorphism and SCC (Figure 1).  Premature births were recorded in 11 patients (4.5%), iron deficiency in 7 (3%) and dermatology symptoms (eczema/rashes) in only 3 patients (1%).

*Table 4. Showing Clinical indications for BTD screening requests*

| Diagnosis Categories | Common presentation | No of Pts | Percent (%) |
|---|---|---|---|
| **Development Delay** | GDD, LD, speech/language delay (SALD), dyslexia and Developmental Coordination Disorder (DCD) | 154 | 63.4 |
| **Social_ASC** | Social communication concerns and ASD | 120 | 49.4 |
| **EBD** | Sleep or behaviour problems including ODD, ADHD, emotional problems (8) and FASD | 107 | 44 |
| **Neurology** | Sensory, visual (including nystagmus) and hearing impairments, Epilepsy, hypotonia, infantile spasm, Microcephaly, Macrocephaly, Dystonia, unsteady gait, gray matter heterotopias, PVL, Ohtahara Syndrome, and Cerebral Palsy. | 53 | 21.8 |

| Genetic syndromes/ Chromosomal defects | Cri du Chat, Klinefelter's syndrome, CNV with micro-duplications, deletions, XYY Syndrome etc | 16 | 6.6 |
|---|---|---|---|
| Congenital defects | Diaphragmatic hernia, dysplastic/duplex kidneys, cleft lip/ palate, talipes, macrognathia, heart defects (including pulmonary atresia, VSD, and Tetralogy of Fallot) | 13 | 5.3 |
| Dysmorphism | Low set ears, slanting palpebral fissures etc | 6 | 2.5 |
| General / other paediatric specialties | Overweight (7), GOR/feeding difficulties (6), asthma (4), other respiratory symptoms (3), PKU (1), pseudo-hypoaldosteronism (1), faltering growth, plagiocephaly etc | 45 | 18.5 |

Legend:

EBD - Emotional and Behavioural Disorders; and Behavioural Disorders; GDD - Global Developmental Delay; LD - Learning Disability; Social_ASC - Social communication/Autism concerns; ODD – Oppositional Defiant Disorder; FASD – Foetal Alcohol Spectrum Disorder; PVL – Peri-ventricular Leucomalacia, CNV – chromosomal Copy Number Variants; VSD – Ventricular Septal Defect; GOR – Gastro-Oesophageal Reflux; PKU – phenylketonuria

**Figure 1.** *Showing Diagnostic Categories for BTD screening requests*
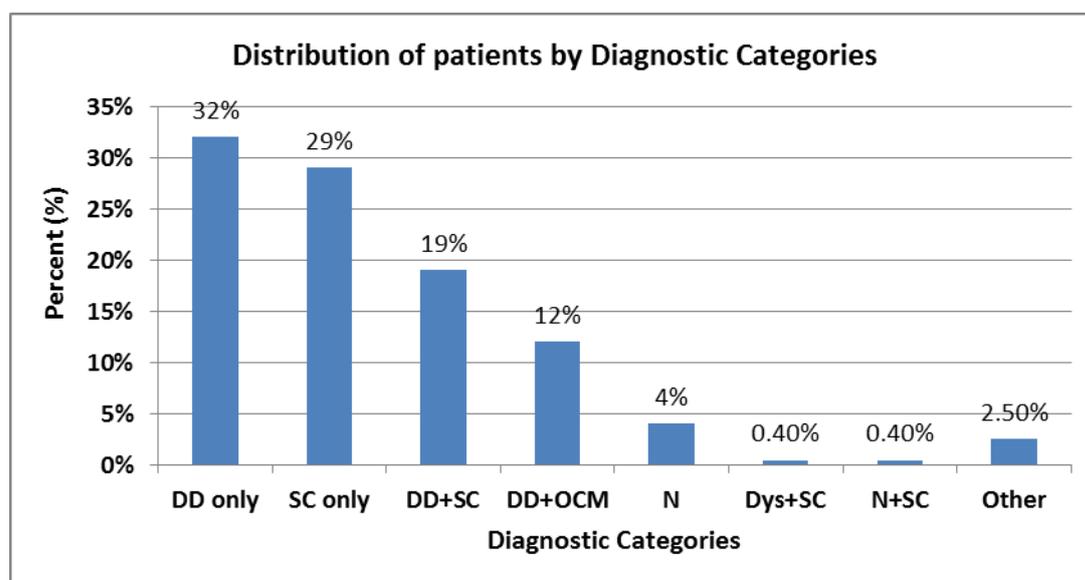


Legend:

DD - Developmental Delay; SC - Social Communication concerns ; N – Neurology; Dys – Dysmorphism; OCM - Other Co Morbidities

### 3.7. Clinical Professionals Requesting BTD Screening Tests

Most of the patients were referred for the BTD tests by ten Community Associate/ Specialist doctors (CASD) (65%) and five Community Consultant Paediatricians (CCP) (28%). This probably reflected higher case load of the CASDs compared to the consultants. Eight Consultant Acute General Paediatricians/Neonatologists (AGPN) referred 7% of the patients. A Child and Adolescent consultant Psychiatrist referred one patient (0.4%) for social communication concerns. Each clinician on average referred 10 patients within the two-year study period (Table 5).

There was a significant difference in the average age and number of diagnosed problems of the patients referred by the different categories of professionals (Table 5).

The Acute (AGPN) and Community consultant Paediatricians (CCP) referred younger children with a mean age of 3 years and 6 mouths compared to the CASDs whose patients were significantly older (seven years old on average). The proportion of CASD's referrals for solitary clinical presentations with either DD and SCC/ASD alone (40.5% and 29% respectively) was significantly higher than that of the CCPs (15% and 34% respectively) and than that of the AGPNs (23.5% and 6% respectively). The CCPs and AGNPs refereed proportionally higher patients with other co-morbidities (49% and 53% respectively) compared to the CASDs (21.5), with chi square p value < 0.001 (Table 6).

*Table 5. Showing the range of Professionals requesting BTD screening tests*

| Clinicians | No Pts | Percent (%) | No Pts per Clinician | Mean Age of Pts (months)$^\$$ | Avg Diag$^\$$ |
|---|---|---|---|---|---|
| Comm_Sp_Doctors | 158 | | 16 | 84.5 ±40.6 | 2.8 ±1.6 |
| Comm_Consultants | 67 | | 13 | 42 ±17.6 | 2.5 ±1.4 |
| Acute/Neon_Consultants | 17 | 7 | 2 | 42.5 ±48.5 | 3.8 ±1.6 |
| CAMHS_Consultants | 1 | 0.4 | 1 | 113 ±0 | 3 ±0 |
| Total | 243 | | 10 | 70 ±42 | 2.8 ±1.5 |
| ANOVS F score | | | | 25.00 | 3.20 |
| P value | | | | <0.001** | 0.024* |

Legend:
Avg_Diag: Average number of diagnosed problems
Comm_Sp_Doc: Community Associate/ Specialist doctors
Comm_Cons: Community Consultant Paediatricians
Acute/Neon: Acute/General and Neonatology
CAMHS: Child and Adolescent Mental Health Service
** Statistically significant

*Table 6. Showing Categories of Diagnosis based on referring Professionals*

| Professional/ Diagnostic categories | DD only | SCC/ASD only | DD+OCM | Others | Total |
|---|---|---|---|---|---|
| Comm_Sp_Ass | 64(40.5%) | 46(29%) | 34(21.5%) | 14(9%) | 158(65%) |
| Comm_Cons | 10(15%) | 23(34%) | 33(49%) | 1(1.5%) | 67(28%) |
| Acute_Neo_Cons | 4(23.5%) | 1(6%) | 9(53%) | 3(18%) | 17(7%) |
| CAMHS | | 1(100%) | 0 | 0 | 1(0.4%) |
| Total | 78 | 71 | 76 | 18 | 243 |
| Percent | 32 | 29.2 | 12.3 | 7.4 | 100 |

(The chi-square statistic is 34.001. The *p*-value is <0.00001)
Legend:
Comm_Sp_Doc: Community Associate/Specialist doctors
Comm_Cons: Community Consultant Paediatricians
Acute/Neon: Acute/General and Neonatology consultants
DD – Developmental Delay; SCC – Social communication concerns; OCM - Other Co-Morbidities (Neurology, Dysmorphism, etc)

### 3.8 Discussion

### 3.8.1. Low positive yield from metabolic screening of children with unexplained neurodevelopmental disorders

This study did not identify any positive case of BTD among 243 children and young people investigated within a Scottish local Authority. Many authors have reported very low positive yield from metabolic screening among children with unexplained neurodevelopmental disorders [9,14,16]. Among a group of 1192 Taiwanese children with developmental delay, only 20.5% (244) had identifiable aetiological risk factors. The rate of abnormalities detection was related to the severity of the disorders [29]. A recent audit of diagnostic investigation among 699 children in a UK tertiary centre reported a total yield of 23.7% from an array of genetic, metabolic and neuroimaging studies. Positive results were more likely to be found among children with developmental delay presenting with additional morbidities. No case of BTD was diagnosed from 227 screening tests over a 6 – year period [30].

The Consultant Paediatricians generally referred proportionally higher patients with other co-morbidities for BTD screen, as opposed to patients with isolated DD or social communication concerns, compared to the Associate/Specialist grade (SASG) Paediatricians. This may be an indication for more regular training and supervision for this cadre of professionals who have proportionately higher clinical caseloads. A recent review of Community Child Health (CCH) services in Scotland confirmed that 77% of the current CCH workforce is SASG doctors and that there has been a 16% fall in the number of Consultant Paediatricians working in CCH since 2007, compared to a 47% increase in Specialist Paediatricians in the acute sector over this period [31].

### 3.8.2. Is Autism an Indication for BTD Screening?

29% of the studied cohort of patients were referred for BTD screening because of concerns about their social communication skills or suspected Autism. Zaffanello et al [32] reported the first case of a 4 year old autistic boy diagnosed with partial BTD but his autistic symptoms did not respond to biotin therapy. His younger brother diagnosed at birth with partial BTD and treated with biotin was asymptomatic. Some authors have reported a higher than normal prevalence of treatable IEM among autistic children, including symptomatic response to treatment with biotin [33]. Among a cohort of 300 Turkish children with ASD, characterized by high consanguinity rates, inherited metabolic disorders (IMD) were diagnosed in nine patients (3%) [34].

Worldwide, the rate of Autism has been steadily rising. There are several environmental factors in concert with genetic susceptibilities that are contributing to this rise. Learning Disability is known to be present in up to 85% of patients with Autism [35] and Fragile X is increasingly identified as causes of Autism, accounting for 2% of cases [36]. This has convinced some authors who have advocated that Autism should also be regarded as a clinical indication for screening children for possible underlying metabolic and genetic disorders [37].

UK National Institute for Health and Care Excellence (NICE) guidelines currently recommend that medical investigations should not be routinely performed for Autism but may be considered on an individual basis that takes into account findings on physical examination, such as dysmorphism and neurocutaneous stigmata, congenital anomalies and intellectual disability or suspicion of epilepsy [38].

### 3.8.3. What Clinical Guidelines Should We Be Using for Metabolic Screening of Children with Global Developmental Delay (GDD) or Severe Learning Disability (LD) in the UK?

First line metabolic screening including assessment of BT enzyme activities is recommended by two evidence-based guidelines for children with significant Developmental Delay (DD) [19,39]. The National Biochemistry Metabolic Network, which is a government (Department of Health) funded organisation [20] however does not approve this approach. Two-tier investigation model offering universal baseline screening to target 60% of treatable IEM with Plasma acylcarnitine profile, amino acids, homocysteine, copper, ceruloplasmin, ammonia and lactate and Urinary creatine metabolites, glycosaminoglycans, oligosaccharides, organic acids, pyrimidines and purines [18], appears to be more cost-effective and financially prudent. A recent analysis of investigations outcome among children with developmental impairments from a single UK tertiary centre confirmed that plasma amino acids and urinary organic acids were among the tests most likely to contribute to a diagnosis of aetiology, with positivity rates of 1.5% and 1.1% respectively [30].

### 3.8.4. Is there a strong case for Newborn Screening of BTD in UK?

There is no universal consensus on the range of disorders to be included in the national Newborn Screening programmes. There is ongoing compelling arguments on either side of the debate about whether BTD screening should be included in the universal Screening programme [5,40]. The advocates for inclusion in the Neonatal Screening schemes argue that subtle neurologic abnormalities may appear as early as at two months of age and that developmental abnormality may occur even in the absence of episodes of overt metabolic decompensation. Furthermore, screening and treatment are both inexpensive and effective, and the incidence of the disease is well within the range of other similar metabolic diseases for which screening is performed in the neonatal period [21]. Even when the test result is inconclusive, the treatment with biotin, which is extremely cheap, can result in unpredictable significant clinical improvement [33,41]. Counterarguments on the other hand insist that the current test is not cost-effective enough, that the true prevalence of BTD is unclear and that further clinical research is required [25].

Few studies have demonstrated the significant cost-effectiveness and clinical benefits of early detection of IMD including BTD, compared to reliance on detection by clinical assessment [42,43]. A study comparing the clinical and neurodevelopmental outcomes in patients with metabolic disorders detected by Expanded Newborn Screening (ENBS) found that 42% of those clinically detected had clinically severe outcomes compared to only 2% of the ENBS-detected cases [44]. Early treatment after Newborn Screening has been reported to prevent occurrence of hearing impairment, optic atrophy with visual loss, delayed motor and speech development in chidren with profound BTD [43]. Excellent outcomes have been reported for older adolescents and adults diagnosed with BTD from Newborn Screening [45].

### 3.8.5. Limitations of the Study

This was a retrospective study and the results need to be interpreted with caution and consideration of its potential flaws. The data used relied on routinely collected documentation in electronic clinical records and laboratory information system. The clinical records might be incomplete and there was no independent verification of the

clinical diagnoses made. There is no record available about the long-term clinical outcomes of the children who were investigated.

None of the 191 patients analysed had abnormal BTA results. However blood samples from a substantial proportion of the patients 53 /243 (22%) could not be analysed due to various specimen- and laboratory-related problems. It is therefore difficult to determine precisely the prevalence of BT deficiency in this small cohort of children and young people.

Considering the estimated incidence of BTD in Europe being 1:47 486 [3], 100% efficient screening of the total NHS Fife local population of 370,330, would be expected to yield only 8 positive cases. It will take over twelve years of 100% efficient screening of all live newborn babies in Fife to detect one case of BTD.

It is difficult to estimate the number needed to screen to prevent death from BTD because no fatality figures have been published. However, it is known that profound BTD may result in coma and death if untreated, and it may be implicated in some cases of sudden infant death syndrome, possibly caused by seizure or brain stem dysfunction [46]. Sudden death at 19 months of age following an insidious presentation of BTD at 14 months has also been reported [47].

## 4. Conclusions

Despite the very low global prevalence of BTD except in certain parts of the world including Turkey and parts of Brazil [48,49], research evidence have pointed to the cost-effectiveness of Neonatal Screening for early detection. BTD is easily treatable and a lifetime of severe irreversible neurological problems, such as developmental delay, hearing loss and optic atrophy, can be averted by universal screen of newborns [1,50]. However Neonatal Screening for profound biotinidase has been reported to produce a high number of false positives [22]. The real economic benefit from the choice of option to screen or not to screen is determined by the balance between the overall cost of a massive screening involving thousands of newborns per year and the cost of more complex medical healthcare regarding much fewer infants clinically diagnosed with BTD [1].

Pilot screening programmes to determine the incidence of BTD are urgently needed to be conducted in the UK, where generalized screening strategies have been excluded because the incidence of BTD is assumed to be too rare [5]. Low positivity rates for BTD screening among children with unexplained neurologic abnormalities or developmental delay does not negate the importance of biotinidase testing in children with clinical patterns specifically suggestive of the deficiency [14].

## Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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