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JAUNDICE IN THE FIRST YEAR OF LIFE

The experience of a Tertiary Hospital

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ABSTRACT

Jaundice, isolated or associated with other signs and/or symptoms, is a challenge to clinical judgement, mainly during the first year of life. It includes multiple pathologies with different approaches and various therapies.

The aim of this study is the characterization of the aetiology, associated clinical manifestations, treatment and follow-up of jaundice cases observed during the first year of life, in *Hospital Pediátrico – Centro Hospitalar de Coimbra*.

The study is based on a retrospective analysis of 480 infants under 12 months (388 newborns and 92 babies) with total bilirubin ≥51,3 μmol/L (3 mg/dl) identified from January 2010 to December 2014.

The median age was 8 days. The majority of infants (40.2%) were admitted in the Emergency Department. The purpose of admission was: jaundice (29.4%), signs of respiratory distress (11%), congenital malformations (9.2%) and gastrointestinal obstruction (7.3%), amongst others. Of the total cohort, 426 (88.8%) showed unconjugated hyperbilirubinemia, largely due to infections/sepsis (n=75), physiological jaundice (n=65), breast milk jaundice (n=63), insufficient breast milk supply (n=56) and prematurity (n=47). Phototherapy was conducted in 102 (23.9%). More than 50% were discharged, 33.1% are followed in outpatient consultation and 4.2% are deceased. Conjugated hyperbilirubinemia was found in 54 and the following diagnoses were preformed: biliary atresia (n=12), infections/sepsis (n=11), idiopathic cholestasis (n=10) and genetic disorders (n=4), amongst others. In this group were performed 15 liver transplants and 6 surgeries (1 Kasai portoenterostomy), the remaining children were hospitalized with medical treatment. About 67% are being followed in outpatient clinic and 18.5% are deceased. One hundred and fifty three (31.9%) presented prolonged jaundice, of which 60 by breast milk jaundice. Statistically significant differences were found between the

presence of infections/sepsis diagnosis and maternal infection history, in the infants with unconjugated ($p<0.05$) and conjugated hyperbilirubinemia ($p<0.01$); and between poor weight gain and hepatomegaly and the presence of cholestasis ($p<0.01$ and $p<0.001$). Age, jaundice duration, gama-glutamyltransferase and alkaline phosphatase values were significantly higher in cholestatic jaundice.

Although most diagnoses are benign, some pathology may be present and must be excluded. The results obtained reinforce the idea that it is mandatory to assess any infant above 14 days that presents jaundice and that the existence of pale stools and/or hepatomegaly are found to be sensitive in liver disease diagnosis. A structured approach is necessary for an adequate and timely response of treatable cases, reason for which an investigative algorithm must be urgently elaborated.

KEYWORDS: jaundice; newborn; infant; aetiology; follow-up.

RESUMO

A icterícia, isolada ou associada a outros sinais e/ou sintomas, constitui um desafio ao raciocínio clínico, principalmente no primeiro ano de vida. Integra múltiplas patologias com abordagens diferentes e terapêuticas variadas.

O objetivo do estudo é a caracterização da etiologia, manifestações clínicas associadas, tratamento e evolução dos casos de icterícia no primeiro ano de vida, observados no Hospital Pediátrico do Centro Hospitalar Universitário de Coimbra.

Baseia-se na análise retrospetiva de 480 crianças com idade inferior a 12 meses (388 recém-nascidos e 92 lactentes) nas quais foi identificada bilirrubinémia total ≥51,3 μmol/L (3 mg/dl), de janeiro de 2010 a dezembro de 2014.

A idade mediana foi de 8 dias. A maioria das crianças (40.2%) foi admitida pelo Serviço de Urgência. O motivo de admissão foi: icterícia (29.4%), sinais de dificuldade respiratória (11%), malformações congénitas (9.2%), obstrução gastrointestinal (7.3%), entre outros. Da amostra total, 426 (88.8%) apresentavam hiperbilirrubinémia não conjugada devida, na sua maioria, a infeções/sépsis (n=75), icterícia fisiológica (n=65), icterícia do leite materno (n=63), hipogalactia (n=56) e prematuridade (n=47). Foi realizada fototerapia em 102 (23.9%). Mais de 50% teve alta, 33.1% são seguidas em consulta e 4.2% faleceram. Em 54 foi encontrada hiperbilirrubinémia conjugada, sendo efetuado o diagnóstico de atrésia biliar (n=12), infeções/sépsis (n=11), colestase idiopática (n=10), patologia genética (n=4), entre outros. Neste grupo, foram realizados 15 transplantes hepáticos e 6 cirurgias (1 portoenterostomia de Kasai), sendo as restantes crianças internadas com tratamento médico. Cerca de 67% são seguidas em consulta externa e 18.5% faleceram. Cento e cinquenta e três (31.9%) apresentaram icterícia prolongada, dos quais 60 por icterícia do leite materno. Foram encontradas diferenças estatisticamente significativas entre a presença de diagnóstico de

infeções/sépsis e história materna de infeções, nas crianças com hiperbilirrubinémia não conjugada ($p<0.05$) e conjugada ($p<0.01$), e entre a má progressão ponderal e hepatomegalia e a presença de colestase ($p<0.01$ e $p<0.001$). Os valores de idade, duração da icterícia, gama-glutamiltransferase e fosfatase alcalina foram significativamente superiores na icterícia colestática.

Apesar da maioria dos diagnósticos serem benignos algumas patologias podem estar presentes e devem ser excluídas. Os dados obtidos reforçam a ideia de que é mandatório avaliar qualquer criança acima dos 14 dias que apresente icterícia e que, a presença de acolia e/ou hepatomegalia são achados sensíveis no diagnóstico de doença hepática. Uma abordagem estruturada é necessária a uma resposta adequada e atempada dos casos tratáveis, pelo que um algoritmo de investigação deve ser urgentemente elaborado.

PALAVRAS-CHAVE: icterícia; recém-nascido; lactente; etiologia; evolução.

INTRODUCTION

Jaundice is the clinical manifestation of elevated serum bilirubin (hyperbilirubinemia) that is characterized by yellow pigmentation of the skin, conjunctivae membranes over the sclera and other mucous membranes.[1,2] It is not a pathologic condition but rather a sign of illness originating from/or affecting the liver and/or blood.[2]

Unconjugated bilirubin (UB), the ultimate breakdown product of haemoglobin, roughly equivalent to indirect-reacting fraction (total minus direct fraction), is conjugated in the liver with glucuronic acid by uridine diphospho-glucuronosyltransferase (UDP-GT) to its water-soluble form, known as conjugated bilirubin (CB) or direct-reacting fraction. This characteristic provides a means to categorize jaundice into two types: unconjugated (UHB) and conjugated hyperbilirubinemia (CHB). The relative proportions of CB and UB are important in establishing aetiology.[2,3]

Jaundice is very common in newborn infants.[3] Approximately 80% of preterm and 50% of term newborns[2,5] have transient jaundice, which usually appears 3 to 5 days after birth and is resolved spontaneously after 1 to 2 weeks. Almost every infant will have a total serum bilirubin level that is above the normal maximum adult level, because of an higher bilirubin production related to increased turnover of erythrocytes and of a transient deficiency in their ability to conjugate (due to immaturity of the hepatic UDP-GT) and clear bilirubin.[4,5] Although mild jaundice is most common among healthy term infants and requires no intervention, it can develop into severe jaundice, associated with *kernicterus* in extremely rare cases. This devastating condition, due to neurological damage caused by UB, leads to death.[6]

Mechanisms that cause UHB include overproduction of heme products or disturbed conjugation. It may be associated with breastfeeding, breast milk, premature birth, hypoxia, extravascular blood (cephalohematoma or bruising), and with other rare causes like drugs,

sepsis, hypothyroidism, hemoglobinopathies, inborn errors of metabolism and gastrointestinal obstruction.[2-4,7]

In contrast, CHB nearly always results from hepatic dysfunction which develops due to many different disorders, including structural abnormalities, such as biliary atresia (BA), bile duct paucity or genetic syndromes, inherited, metabolic or endocrine disorders and other miscellaneous causes.[4,8]

Serum levels of bilirubin must exceed 42.8/51.3µmol/L (2.5/3mg/dL) for jaundice to become evident. The threshold, however, can vary by age group, ranging from 85.5-119.7µmol/L (5-7mg/dL) in the newborn, to above 34.2µmol/L (2mg/dL) in the older child. In healthy children, most bilirubin circulates in its unconjugated form; less than 5% of circulating bilirubin is present as CB.[2,7,9]

Icterus may be the first and sometimes only sign of elevated bilirubin levels but in some, more severe, cases the infant may also appear sleepy, lethargic, mild hypotonic, with poor sucking, with a high-pitched cry, or reveals hepatomegaly on examination. A history of persistently yellow urine or pale stools confirms the presence of CHB.[3,8]

Factors associated with severe hyperbilirubinemia [total bilirubin ≥200µmol/L (11.7mg/dL)][4] include Maternity Unit pre-discharge, total serum or transcutaneous bilirubin measurement in the high-risk or high-intermediate-risk zone, exclusive breastfeeding (particularly if weight loss is excessive), low birth weight, prematurity, jaundice observed in the first 24 hours of life, additional symptoms and signs such as dark urine or pale stool, prolonged jaundice, maternal diabetes mellitus and cephalohematoma or significant bruising.[3-5,8,10]

The differential diagnosis of a jaundiced baby is a challenge to clinical judgment considering the multiplicity of situations that may occur or develop simultaneously with this sign. The aim

of this study is the characterization of the aetiology, associated clinical manifestations, treatment and follow-up of jaundice cases observed during the first year of life, in *Hospital Pediátrico – Centro Hospitalar de Coimbra* (HP-CHUC).

MATERIALS AND METHODS

The sample of this retrospective study was collected from the Laboratory database of HP-CHUC, between the 1st of January 2010 and the 31st of December 2014 (5 years). The collected parameters were: clinical file number, age, harvest sample date and total and direct bilirubin levels.

By applying a less than 12 months of age filter to a 5342 cohort, a sample of 2894 was obtained. From these were selected those with a bilirubin level equal or above 51.3 µmol/L, in a total of 1680. After removing the duplicated clinical files, a final sample of 522 (418 newborns and 104 babies) was obtained.

During data collection 42 other samples were excluded: 12 for having a haemolysed, non available or insufficient blood sample and 30 for being the patient´s only harvested sample. In the process file of these samples there was no reference to jaundice or relevant pathology.

The following information was collected from the files of the final group of 480 patients: age at harvest date, gender, race, admittance origin, cause for admission/transfer, analytic parameters [total and direct bilirubin, gama-glutamyltransferase (GGT), serum alkaline phosphatase (ALP), haemoglobin and blood type], gestational age and birth weight, the need for post-childbirth reanimation, phototherapy history, breastfeeding history, jaundice duration, physical examination (prostration and hepatomegaly), associated signs and symptoms [vomiting, poor weight gain (PWG), dark urine and pale stools], family history, clinical diagnosis, therapy and follow-up.

The data collected was obtained accessing the online platforms *SClínico* and *Plataforma Dados de Saúde* through file case numbers associated to the selected harvest.

Other data was extrapolated: prolonged jaundice (>14 days in term infants and >21 days in preterm infants), prematurity (<37 weeks' gestational age), intrauterine growth restriction (IUGR, having as reference World Health Organization's percentile curves) and the distinction between UHB and CHB. Cholestasis was defined as jaundice, present at any moment after birth, with CHB exhibiting a CB >51.3 µmol/L [3 mg/dl, in combination with a total bilirubin of <256.5 µmol/L (<15 mg/dL) or a CB fraction of >20% of the total].[10]

The data gathered was registered and encoded in Microsoft Excel 2007®. Analyses were performed with the use of IBM software – SPSS (Statistical Package for the Social Sciences) for Mac®, version 22.0 (IBM®). All reported P values are two-tailed, with the P value of 0.05 indicating statistical significance. Categorical variables are presented as frequencies and percentages, and continuous variables as means and standard deviations (SD), or medians and inter-quartile ranges (IQR) for variables with skewed distributions. Normal distribution was checked using Shapiro-Wilk test or skewness and kurtosis. Categorical variables were compared with the use of Fisher's exact test or the Chi-square test, as appropriate. Continuous variables were expressed as means ± SD and compared with the use of Student's T-test or as medians and IQR and compared with the use of the Mann-Whitney test.

RESULTS

Of the total cohort (N = 480), 388 correspond to newborns and 92 to babies. The sample distribution throughout the 5 years, according to CHB and UHB results, is represented in Figure 1.

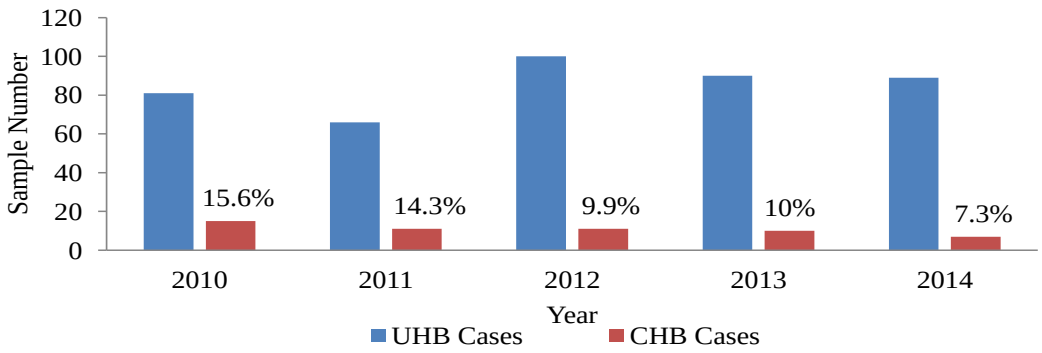

Figure 1 - Sample distribution per year, according to unconjugated hyperbilirubinemia (UHB) and conjugated hyperbilirubinemia (CHB) results

The median age was 8 days with an IQR of 20 (the minimum was 0 and maximum was 347). Sixty two percent of infants were male.

The patients were mainly admitted to the Emergency Department (ED, 40.3%), either from the exterior (22.3%), the General Practitioner (15.7%) or from the patients' referral phone service *Saúde 24* (2.3%).

The remaining cases were transferred from another National Hospital (38.3%) or from Coimbra's Maternity Units (15.8%), admitted in outpatient consultations (5.2%) or, in a smaller number, from a foreign hospital (0.4%).

The motives for patients' admission or referral (Fig.2) were varied, being jaundice the most prevalent one (n=141, 29.4%).

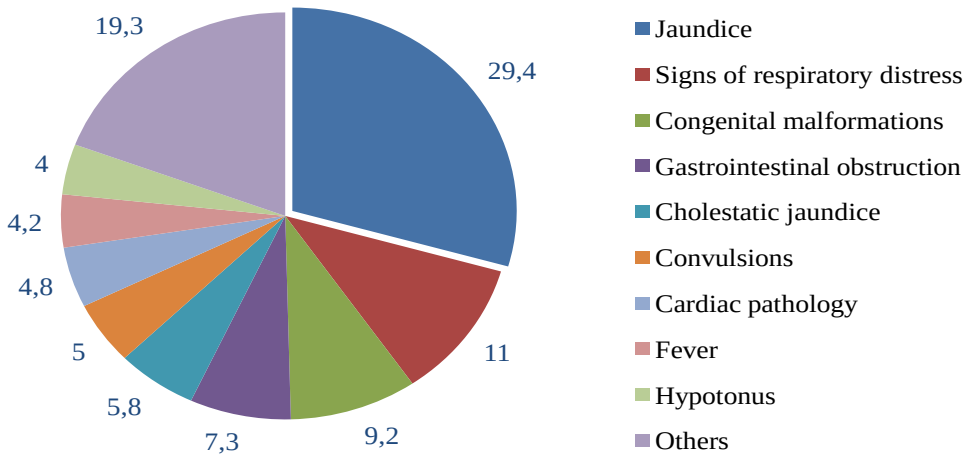

Figure 2 – Purpose (%) for admission or referral of patients (N=480)

Non cholestatic jaundice

Non cholestatic jaundice was found in 426 patients (88.8%) of whom the majority were male (63.8%) and Caucasian (97.7%). The median age, at the moment of blood collection, was of 4 days (from 0 to 330, IQR 12). The greater part was term infants (70.7%) with a median gestational age of 38 weeks (25 to 42, IQR 3), a median birth weight of 2886±741g and largely without need for post-childbirth reanimation (69.7%).

More than half (57.7%) was exclusively on breastfeeding. Previous phototherapy was reported in 96 (22.5%) infants and PWG was observed in 34%.

Regarding family history, 72.1% had no known diseases, 8.2% had sexually transmitted infections or presented other infections during pregnancy, 6% had gestational diabetes mellitus, 3.3% had hereditary haematological diseases and 1.6% was consanguineous.

Although physical examination was normal in the majority, prostration was observed in 28.9% and hepatomegaly in 5.2%. Five cases presented dark urine (2 hereditary spherocytosis, 1 autoimmune haemolytic anaemia, 1 prematurity and 1 breastfeeding).

More than 59% (n=252) of the baby's blood types were unknown. Overall Rhesus positive type totalled 35.2% (n=150). The most common were A+ (n=79, 18.5%) and 0+ (n=55, 12.9%). Other laboratory results (total and direct bilirubin, ALP, GGT and haemoglobin) are summarized in Table 1.

Table 1 – Laboratory results from infants with unconjugated hyperbilirubinemia (n=426)

Total bilirubin (μmol)	**180.7** (23.8-489, IQR 117.6)
Direct bilirubin (μmol)	**0** (0-46.5, IQR 0)
GGT (UI)	**101** (IQR 91)
ALP (UI)	**157** (IQR 149)
Haemoglobin (g/dL)	**14.6** ± 2.9

Gama-glutamyltransferase (GGT); alkaline phosphatase (ALP)

The main diagnoses were: infections/sepsis (n=75, 17.6%), physiological jaundice (n=65, 15.3%), breast milk jaundice (n=63, 14.8%), insufficient breast milk supply (n=56, 13%) and prematurity (n=47, 11%). From the remaining cases (28.3%) some must be highlighted: hematologic causes (n=17), cephalohematoma (n=15), congenital hypothyroidism (n=8) and metabolic disorders (n=5).

Most patients didn't require therapeutic intervention (n=115, 27%). Phototherapy was performed in 102 babies (23.9%) of whom 32 needed other interventions such as: surgery (12 gastrointestinal obstruction), intensive care management (3 hypoxia, 6 infections),

supplementation with formula milk (7 insufficient breast milk supply) and medical therapies (1 congenital hypothyroidism, 1 hypopituitarism, 1 methylmalonic acidemia and 1 infection).

In the Intensive Care Unit (ICU) were admitted 25.4%. One of the patients, with a mitochondrial disease (DGUOK deficiency), was submitted to a liver transplant due to acute liver failure.

Regarding follow-up, 51.4% were discharged, 33.1% are being followed in outpatient consultation in HP-CHUC and 11.3% were transferred to their regional reference Hospital. Eighteen patients (4.2%) died: 10 due to multiorgan failure (four of them with sepsis), 3 during surgery (cardiac and neurologic pathology), 2 with perinatal asphyxia, 2 with inherited metabolic diseases (a CDG-Congenital Disorder of Glycosilation and the DGUOK deficiency patient) and 1 from sudden death during hospitalization (a preterm infant with oesophagus atresia).

Cholestatic jaundice

Of the case sample with cholestatic jaundice (n=54), 50% were male and 14.8% (n=8) African, being the remaining Caucasian. Two of the African infants were referred from a native Hospital to the hepatic transplant center. The median age at the moment of blood collection was of 49 days (from 3 to 347, IQR 95). The majority were term infants (72%) with a median gestational age of 38 weeks (24 to 41, IQR 3) and mean birth weight of 2634±909g. Post-childbirth reanimation was necessary in 27.8% (n=15). Of the 42 babies under 120 days, only 23.1% were fed exclusively on maternal milk. Previous phototherapy was reported in 21 infants (38.9%).

Family history was unremarkable in 66.7%, 16.7% had sexually transmitted infections or infections during pregnancy and 3.7% gestational diabetes mellitus. There was no consanguinity in this group.

On physical examination 63% presented hepatomegaly and 38.9% prostration. PWG was observed in 57.4%, pale stools in 42.6%, dark urine in 29.6% and vomiting in 20.4%.

Baby's blood types were known in 83.3%. Overall Rhesus positive type totalled 75.9% (n=41). The most common were A+ (n=18, 33.3%) and 0+ (n=17, 31.5%). Other laboratory results (total and direct bilirubin, haemoglobin, ALP and GGT) are summarized in Table 2.

Table 2 – Laboratory results from infants with conjugated hyperbilirubinemia (n=54)

Total bilirubin (μmol)	**116.7** (51.5-525.4, IQR 109.7)
Direct bilirubin (μmol)	**54.3** (15.9-372.4, IQR 60.58)
GGT (U/I)	**159** (IQR 324)
ALP (U/I)	**457** (IQR 503)
Haemoglobin (g/dL)	**11.3** ± 3.2

Gama-glutamyltransferase (GGT); alkaline phosphatase (ALP)

The diagnoses were varied (Fig. 3). From the 12 cases of BA, 2 came to HP-CHUC for pre-transplantation assessment with the etiologic diagnosis already established.

Cholestasis in the context of infection was multi-factorial: gastrointestinal perforation (n = 4), cardiac and renal malformations (n=2), *Escherichia coli* septicemia (n=1), congenital syphilis (n=1), HIV maternal infection (n=1), meconium aspiration syndrome (n=1) and fever without cause (n=1). It was mostly transient, ceasing when the aetiology was treated. In 3 infants, however, the outcome was death: pyelonephritis and acute renal failure in a child with pyelocalyceal malformation, *Escherichia coli* septicemia and maternal HIV infection.

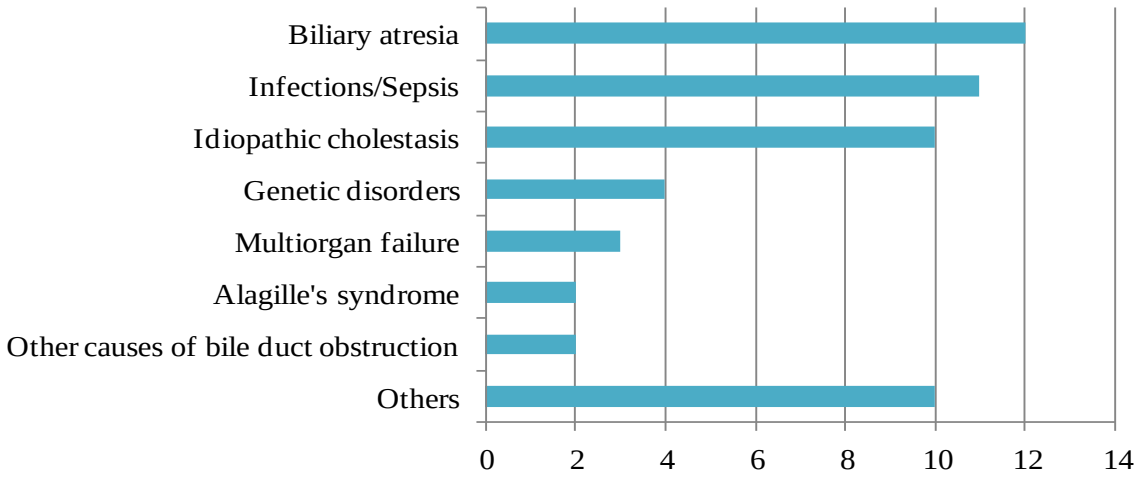

Figure 3 – Diagnoses of conjugated hyperbilirubinemia (n=54)

In this group were performed 15 liver transplants and 6 surgeries (1 portoenterostomy of Kasai). The remaining children were hospitalized with medical treatment. Only 2 conducted phototherapy (sepsis diagnosis).

About 67% of the patients are followed in outpatient clinic (in one or several specialties of HP-CHUC), 11% were discharged and 3.5% were transferred to their regional reference Hospital. The mortality rate was of 18.5% corresponding to 8 cases with multiorgan failure (6 septic shock and 2 indeterminate causes), 1 of perinatal asphyxia and 1 genetic disorder.

Prolonged jaundice

Considering the criteria of prolonged jaundice (>14 days in term infants and >21 days in preterm infants), 153 cases (31.9%) were selected, 113 non cholestatic and 40 cholestatic. Ninety-four were male (61.4%). The median gestational age was of 38 weeks (24-41, IQR 2) and the weight of 3020g (910-4445, IQR 870).

The median age and the median value of total bilirubin at admission was of 33 days (15-347, IQR 33) and of 160.9µmol (51.5-525.4, IQR 114.6), respectively. From the studied group, 106 (69.3%) fed exclusively on breast milk and 52 (34%) had had previous phototherapy.

The causes associated to prolonged jaundice were: breast milk (n=60), sepsis (n=40), insufficient breast milk supply (n=16), BA (n=11), idiopathic (n=9) and haematological (n=8), amongst less frequent others. Of the 63 cases diagnosed with breast milk jaundice (in the total sample), 95% (n=60) presented prolonged jaundice.

In the prolonged unconjugated jaundice subgroup (n=113), 67% were male. The median gestational age was of 38 weeks (26-42, IQR 2) and the mean birth weight was of 3023±564g. On admission, the mean value of total bilirubin was of 179.7±69.2µmol. About 83% were premature and 4% presented IUGR. Ninety-seven infants (85.8%) fed exclusively on breast milk.

Regarding family history, it must be highlighted that in 7 infants the mothers have had infections during pregnancy or during peripartum and 4 had gestational diabetes mellitus. The clear majority of family histories were not relevant.

The most frequent diagnoses were: breast milk (n=60), sepsis (n=18), insufficient breast milk supply (n=16) and haematological diseases (n=7).

Motives for admission or referral of patients

By analysing the cases whose purpose for consultation was *jaundice* (n=141), 84% came to HP-CHUC via ED, half of which were referred by their General Practitioner or by *Saúde 24*. The remaining cases came transferred from another Hospital, Maternity Units or were observed in consultation.

The diagnoses of these cases were mostly benign: breast milk jaundice (n=50, 35.5%), insufficient breast milk supply (n=32, 22.7%), physiological jaundice (n=31, 22%) and prematurity (n=13, 9.2%). Three infants, however, showed cholestatic jaundice. One diagnosed with idiopathic cholestasis and 1 with alpha-1-antitrypsin deficiency which required liver transplant (both transferred from another Hospital). The third infant arrived from the exterior to the ED and was diagnosed with BA. Was then, submitted to a Kasai procedure without successful outcome that led to liver transplant. The 3 infants are nowadays being followed at hepathology consultations.

The vast majority of the 141 cases were discharged (87.2%), although one patient's death, due to post mortem diagnosis of a metabolic disorder (Golgi's Complex), must be noted.

Considering the admission for *cholestatic jaundice* (n=28) the diagnoses were: BA (n=9), idiopathic cases (n=8), metabolic disorders (n=3), Allagile's syndrome (n=2), type 4 progressive familial intrahepatic cholestasis (n=1), Caroli disease (n=1), choledochal cyst (n=1), Rh isoimmunization by in uterus transfusions (n=1), alpha-1-antitrypsin deficiency (n=1) and genetic syndrome (n=1).

The patients were referred from other Hospitals (national and African), and from HP-CHUC outpatient clinic. The majority was submitted to medical therapeutic (n=11) or liver transplantation (n=10) and are now being followed in specialty consultation. Only two deceased, both without definitive diagnosis, one with storage disease hypothesis and another with choledochal cyst rupture hypothesis which had been previously submitted to a Kasai portoenterostomy in another Hospital.

In the 311 patients with *other reasons* for evaluation, excluding jaundice and cholestatic jaundice, the majority presented UHB (n=287, 92.3%), mostly with diagnoses of sepsis (n=65, 20.9%), prematurity and other causes associated to prematurity (n=68, 21.9%). These

patients were, largely, transferred from other Hospitals (n=148, 47.6%), Maternity Units (n=72, 23.2%) or referred by their General Practitioner (n=16, 5.1%).

From the 24 cases that presented CHB, 87.5% came from Maternity Units or other Hospitals with suspicions of gastrointestinal obstruction (n=9), hemodynamic instability (n=5), congenital malformations (n=4), haematological disease (n=1), or for pre-transplant assessment (n=2); the remaining 3 came to ED due to fever, refusal to feed and diarrhoea/vomiting. Diagnoses of fever without cause, congestive heart failure (in a baby with known cardiac pathology) and acute gastroenteritis were performed.

Infections/Sepsis Diagnosis

Regarding the patients with infections/sepsis diagnosis (n=86) the majority presented UHB (87.2%) and were of term (68.6%) Most of them without urinary tract infection (60.5%) nor IUGR (90.7%). In approximately 18% there was reference of peripartum maternal infection, positive *Streptococcus* group B screening or sexually transmitted infections (HIV and HCV).

Statistical analyses

There is a statistical significance between the presence of infection/sepsis diagnoses and the maternal history of infection, both in infants with CHB (Fisher´s exact test, $p<0.01$) as in infants with UHB (Chi-square test, $p<0.05$) (Table 3).

Table 3 – Comparing infections/sepsis diagnosis and other diagnoses with infectious and non-infectious maternal history, in conjugated hyperbilirubinemia (CHB, n=54) and unconjugated hyperbilirubinemia (UHB, n=426) groups

CHB		**Maternal history**		
		Infectious	Non-infectious	
Diagnoses	Infections/Sepsis	6	6	
	Others	38	4	p=0.005*
UHB		**Maternal history**		
		Infections	Non-infectious	
Diagnoses	Infections/Sepsis	11	67	
	Others	24	324	p=0.036**

All data are expressed as n; * Fisher´s exact test ** Chi-square test

There is a statistically significant association between PWG, hepatomegaly, pale stools and the presence of cholestasis (Chi-square test, $p<0.01$, $p<0.001$ and $p<0.001$, respectively) (Table 4).

Table 4 – Comparing some physical examination parameters [poor weight gain (PWG), hepatomegaly, pale stools, prostration] and previously phototherapy with unconjugated hyperbilirubinemia (UHB, n=426) and conjugated hyperbilirubinemia (CHB, n=54)

	UHB (n=426)	CHB (n=54)	Chi-square test
PWG	145	31	p=0.01
Hepatomegaly	22	34	p<0.01
Pale stools	3	23	p<0.01
Prostration	123	21	p=0.130
Previous phototherapy	96	21	p=0.348

All data are expressed as n

The patients with cholestasis presented significantly higher values of age, jaundice duration, GGT and ALP, comparatively to the patients without cholestasis (Mann-Whitney test, all with p<0.01). Furthermore the gestational age values were not significantly different between the groups (Mann-Whitney test, p>0.05) (Table 5).

Table 5 – Comparing age, gestational age, jaundice duration and some laboratorial results [gama-glutamyltransferase (GGT), serum alkaline phosphatase (ALP)] in unconjugated hyperbilirubinemia (UHB, n=426) and conjugated hyperbilirubinemia (CHB, n=54)

Medians	UHB (n=426)	CHB (n=54)	Mann-Whitney test
Age (days)	**7** (IQR 14)	**49** (IQR 95)	p<0.001
Gestational age (weeks)	**38** (IQR 3)	**38** (IQR 3)	p=0.756
Jaundice duration (days)	**3** (IQR 10)	**24** (IQR 74)	p<0.001
GGT (U/I)	**101** (IQR 91)	**159** (IQR 324)	p<0.001
ALP (U/I)	**157** (IQR 149)	**457** (IQR 503)	p<0.001

Within the CHB group (Table 6) there is no statistically significant association between BA and IUGR (Fisher's exact test, p>0.05) nor between BA diagnosis and PWG (Chi-square test, p>0.05). The mean birth weight in the BA group isn't significantly different from the other diagnoses group (T-student test, p>0.05), nor the median gestational age in the BA group (38.5 weeks, IQR 1) is significantly different (Mann-Whitney test, p>0.05), from the median gestational age of the other diagnoses group (38.0 weeks, IQR 4).

Table 6 – Comparing some initial clinical parameters [intrauterine growth restriction (IUGR), poor weight gain (PWG)], gestational age and birth weight in infants with biliary atresia (BA, n=12) or other disorders (n=42) causing neonatal cholestasis (n=54)

CHB	BA (n=12)	Others (n=42)		Test
IUGR (n)	3	9	p=0.682	Fisher´s
PWG (n)	6	6	p=0.556	Chi-square
Gestational age (weeks)	**38.5** (IQR 1)	**38.0** (IQR 4)	p=0.134	Mann-Whitney
Birth weight (g)	**2993**±779	**2521**±928	p=0.119	T-student

Conjugated hyperbilirubinemia (CHB)

DISCUSSION

The distribution of cases per year followed a uniform distribution, although in 2010 there were about twice as many CHB cases as in 2014. This declining trend has been relatively consistent over the years. (Fig.1).

As expected, the majority of the infants was observed during the neonatal period, being the non cholestatic jaundice the most prevalent.[4] Despite the main causes being *benign* (physiological jaundice, breast milk jaundice, insufficient breast milk supply and prematurity), as observed in other studies[11] some pathologies can be identified and should be excluded, especially if alert signs or symptoms are present.

The immediate priority in a jaundiced child is to differentiate between cholestasis, generally pathologic, and the unconjugated hyperbilirubinemia.

In the treatment of children with UHB, when indicated and when there are no criteria for phototherapy, other alternatives to hospitalization should be considered (support or supplementation with adapted milk).[5] The therapeutic approaches referenced in this study followed this indication, since it was recommended to monitor the alert signs and to initiate supplementation with formula milk to a large part of the children. However, although rare, more severe pathologies have been identified, namely endocrine, requiring specific medical treatment.

The number of cases with cholestasis found in this study was higher than in other descriptions in literature, as in the Saudi Arabia[11] study (11.2 vs. 4%). This disparity may be explained by the fact that we studied a population of a hospital with a reference liver transplant Paediatric center and because our study had a larger age range (347 vs. 60 days).

Alkhotami[11] found a statistically significant difference in age between the groups with cholestatic and non-cholestatic jaundice, a result also found here. In fact, in our cohort, the diagnosis in patients with cholestatic jaundice was done later and in the context of prolonged jaundice (Table 5). Nowadays, it is actually mandatory to assess any neonatal jaundice lasting more than 14 days[4,10,12] in term infants and than 21 days in preterm infants. As described in the literature[8] and proven in this study, even before any laboratory evaluation, the presence of pale stools and/or hepatomegaly are very sensitive clinical clues for liver disease (Table 4).

Regarding pale stools, it's important to highlight that parents are not always capable to distinguish normal color from pale stools, especially in a first child. Some countries have campaigns to promote early diagnosis and appropriate referral for liver disease in newly born infants. In England, the *Children's Liver Disease Foundation* developed the free *Yellow Alert app* in order to make the early detection of alert signs easier for parents. It includes: information regarding the signs of liver disease in newborns and a stool chart allowing users to compare a newborn's stool to a variety of healthy and suspicious colours.[13]

In patients with cholestatic jaundice, we found a wide variety of diagnoses, with BA being the most common (22%, Fig. 3). The same was reported by Hoerning[10] in a study of a tertiary paediatric center in Germany where, in a total of 82 infants with cholestasis, 41% were BA (Table 7). This reinforces the relevance of having a structured approach investigation in order to promptly detect potentially treatable causes.[8] In the BA cases, it's important to have a rapid definitive diagnostic so that Kasai procedure is not delayed (which would jeopardize the outcome and final prognosis).[4,10]

Cholestatic jaundice of unknown cause (*idiopathic*) is also very prevalent in all three studies[10,14] and can be overestimated. On the other hand, the percentage of cholestasis in the

context of infections/sepsis was much more prevalent in this study than what was found by Lee in a study conducted in Malaysia (Table 7).[14]

In the particular case of infections diagnosis, the presence of maternal and/or peripartum infections was a risk factor (Table 3). Cholestasis in this context was multifactorial and mostly transient.

Table 7 - Most common disorders causing cholestasis in this study compared to a study from Germany[10] and a study from Malaysia[14]

Study	n	Country	BA (%)	IC (%)	Infections (%)	Metabolic, Endocrine, Genetic (%)	PFIC, Allagile (%)	Others (%)
HP-CHUC	54	Portugal	22	19	20	8	6	25
Hoerning[10]	82	Germany	41	13	1	9	12	24
Lee[14]	146	Malaysia	29	38	14	1	5	13

In the German study, 30 of the 82 cholestatic cases required liver transplant (~37%), a higher value than that found in our study (~28%) probably related to a higher number of BA. On the other hand, mortality was slightly higher in our cohort (18.5 vs. 12.5%), mainly due to cases of multiorgan failure in children diagnosed with septic shock whereas in the German study the deaths were mainly in patients diagnosed with BA.[10] Though, both in our study and that of Hoerning's[10] there was no statistically significant association in what concerns mortality rate and number of liver transplants ($p>0.05$ and $p>0.05$, Chi-square test).

Prognosis is associated to its underlying disorder. Mortality in this study was mainly related to multiorgan failure in patients with sepsis or perinatal asphyxia. Most cases of idiopathic cholestasis can regress along time.[8]

As this study represents the experience of a single tertiary hospital, it cannot be assumed as a comprehensive investigation of jaundice in the first year of life. That fact and the retrospective feature of this investigation, dependent on the amount of information available in the clinical files, are its major limitations.

In conclusion, jaundice is very common, especially in newborn babies and is frequently uncomplicated and transitory. Nevertheless, this study highlights the importance of excluding pathologic causes, mainly if prolonged jaundice and/or cholestasis are present. In these cases, an investigative algorithm should be urgently performed.

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