Contributo para a melhoria da acuidade do diagnóstico pré-natal: análise crítica da correlação entre o diagnóstico pré-natal e o diagnóstico *post-mortem*

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…

Álvaro de Campos
in Tabacaria

Abreviaturas

A	Adenina
ACOG	*American College of Obstetricians and Gynecologists*
APRV	*Anomalous pulmonary venous return*
Array-CGH	A*rray Comparative Genomic Hybridization*
AS	*Aortic stenosis*
AV	*Auriculo-ventricular*
AVSD	*Atrioventricular septal defects*
BPD	*Biparietal diameter*
C	Citosina
COA	*Coarctation of aorta*
COA-IVS	*Coarctation of aorta with intact ventricular septum*
CMP	*Cardiomyopathy*
CMV	*Human cytomegalovirus*
CTG	Citosina - timina – guanina
Del	Delecção
Der	Cromossoma derivado
DISV	*Double inlet single ventricle*
DOLV	*Double outlet left ventricle*
DORV	*Double outlet right ventricle*
DMR	*Differentially Methylated Regions*
DNA	Ácido desoxirribonucleico
DS	*Down syndrome,* síndrome de Down
EUA	Estados Unidos da América
FD	*Fetal death*
FISH	*Fluorescent In-Situ Hybridization*
G	Guanina
hCG	Hormona gonadotrofina coriónica
HHV-6	*Human Herpesvirus 6*
HLHS	*Hypoplastic left heart syndrome*
QF-PCR	Q*uantitative Fluorescent Polymerase Chain Reaction*
IAA	*Interrupted aortic arch*
IMG	Interrupção médica da gravidez
ISUOG	*International Society of Ultrasound in Obstetrics and Gynecology*
KCl	Cloreto de potássio
LVOTO	*Left ventricular outflow tract obstruction*
MA	*Mitral atresia*
mat	Origem materna
MeDiP	Imunoprecipitação do DNA metilado
MLPA	*Multiplex Ligation-dependent Probe Amplification*
NBL	*Nasal bone length*
OFD	*Occipito-frontal diameter*

OMIM	*Online Mendelian Inheritance in Man*
PA	*Pulmonary atresia*
PAPP-A	Proteína plasmática associada à gravidez
PRM	*Premature rupture of membranes*
PS	*Pulmonary stenosis*
PVS	*Pulmonary valve stenosis*
RCOG	*Royal College of Obstetricians and Gynecologists*
RVOTO	*Right ventricular outflow tract obstruction*
SD	*Standard deviation*
SMLV	*Single morphologically left ventricle*
T	Timina
TA	*Tricuspid atresia*
TGA	*Transposition of the great arteries*
TOF	*Tetralogy of Fallot*
TOP	T*ermination of pregnancy*
uE3	Estriol não conjugado
VA	*Ventriculo-arterial*
VSD	*Ventricular septal defect*

Resumo

Summary

Resumo

O diagnóstico pré-natal e a interrupção médica da gravidez são actividades em que a avaliação da qualidade é crucial. Vários métodos de avaliação têm sido definidos, baseados sobretudo na avaliação da qualidade da ecografia pré-natal. Um outro processo de efectuar a avaliação de qualidade do diagnóstico pré-natal é a comparação entre o diagnóstico pré-natal e o diagnóstico *post-mortem*, nos casos em que a gravidez é interrompida.

No **Capítulo 3** foi efectuada a **correlação entre o diagnóstico pré-natal e o diagnóstico *post-mortem*** nas situações de interrupção médica da gravidez de causa fetal. A comparação entre os achados pré-natais e os achados *post-mortem* mostrou uma concordância total em 61,1% dos casos, e não houve nenhum caso de discordância total. A autópsia permitiu alterar o risco de recorrência em 32% dos casos em que acrescentou informação.

No **Capítulo 4** foi avaliado o **contributo da autópsia nos casos de interrupção médica da gravidez por anomalias cromossómicas**. Neste tipo de anomalias o diagnóstico definitivo já está estabelecido, mas a autópsia detectou anomalias *major* não previamente identificadas em 12,2% dos casos e em 14% uma anomalia detectada na ecografia não foi confirmada. O grupo de anomalias em que se observou maior acréscimo de informação foi no grupo das anomalias cardíacas, pelo que a realização de ecocardiograma previamente à decisão de interrupção de gravidez se torna indispensável.

No **Capítulo 5** procedeu-se à avaliação da **variabilidade fenotípica na síndrome de Down**. Apesar do fenótipo da síndrome de Down poder ser, em parte, reconhecido a partir das 14 semanas, um feto no 2º trimestre pode ainda não ter definidas as alterações características, parecendo normal mesmo a observadores experientes. No estudo caso-controlo efectuado, os parâmetros biométricos que apresentavam diferenças com significado estatístico foram apenas o diâmetro occipito-frontal e o comprimento do osso do nariz. Encontramos anomalias craniofaciais em 96% dos fetos com síndrome de Down e quando avaliamos as anomalias craniofaciais isoladamente, encontramos diferenças com significado estatístico em todas excepto no tamanho das orelhas.

No **Capítulo 6** avaliamos o **contributo da autópsia perinatal nas anomalias cardíacas congénitas**. Foram detectadas anomalias cardíacas em 13,6% dos fetos submetidos a autópsia. Nestes fetos foram detectadas anomalias associadas em 67,7% dos casos. Nas anomalias cardíacas, a avaliação da concordância entre os achados pré-natais e *post-mortem* mostrou uma discordância total em 8% dos casos. Quando a avaliação pré-natal foi efectuada por um cardiologista pediátrico a discordância foi significativamente menor.

Em conclusão, a realização sistemática da autópsia em situações de interrupção médica da gravidez permite, em muitos casos, estabelecer o diagnóstico definitivo e redefinir o prognóstico, contribuindo para um adequado aconselhamento dos casais (Capítulo 3 e 6).

Nos casos das anomalias cromossómicas a autópsia permite detectar anomalias que podem contribuir para uma melhor caracterização morfológica e conhecimento da história natural das aneuploidias em idades gestacionais precoces (Capítulo 4 e 5).

No caso particular da síndrome de Down, um melhor conhecimento do fenótipo fetal pode ajudar a definir novos marcadores ecográficos que contribuam para melhorar a capacidade de identificação pré-natal desta síndrome (Capítulo 5).

A autópsia com avaliação exaustiva do coração é importante para a detecção e melhor caracterização morfológica das anomalias cardíacas (Capítulo 4 e 6).

Summary

Prenatal diagnosis and medical termination of pregnancy are activities in which quality assessment is crucial. Various methods of quality assessment have been defined, based mainly on assessing the quality of ultrasound. Another process to assess the quality of prenatal diagnosis is the comparison between prenatal diagnosis and post-mortem diagnosis.

In **Chapter 3** the **correlation between prenatal diagnosis and post-mortem diagnosis** was made in cases of elective termination of pregnancy due to fetal causes. The comparison between the pre-natal and post-mortem findings showed complete agreement in 61.1% of the cases, and there was no case of total disagreement. The autopsy resulted in the change of the risk of recurrence in 32% of cases.

In **Chapter 4** the **contribution of the autopsy in cases of termination of pregnancy due to chromosomal abnormalities** was evaluated. In these cases of termination of pregnancy there is already a definitive diagnosis and therefore autopsy does not alter the primary diagnosis or its implications. But the autopsy identified major anomalies not previously recognized in 12.2% of cases, and in 14% the anomaly previously detected by ultrasound was not confirmed. Cardiac anomalies was the group of anomalies in which there was a greater increase in information. Therefore, it is important to perform an echocardiogram before the couples decide to interrupt the pregnancy.

In **Chapter 5** the **phenotypic variability in Down syndrome** was evaluated. The phenotype of Down syndrome can be recognized in a fetus as early as 14 weeks' gestation but fetuses in the second trimester may have not yet developed the typical abnormalities; indeed, some fetuses appear normal even to the experienced observer. In this case-control study, the only biometric parameters significantly different were the occipito-frontal diameter and nasal bone length. Craniofacial anomalies were found in 96% of fetuses with Down syndrome and when evaluated separately, small ears were the only craniofacial feature not significantly different.

In **Chapter 6** the **contribution of perinatal autopsy in congenital heart anomalies** was evaluated. Abnormal cardiac findings were identified in 13.6% fetuses submitted to autopsy. Associated anomalies were found in 67.7% of fetuses with cardiac anomalies. In cardiac anomalies, the correlation between the pre-natal and post-mortem findings showed disagreement in 8% of cases. When the prenatal evaluation was performed by a pediatric cardiologist there was less disagreement.

In conclusion, the systematic autopsy in cases of medical termination of pregnancy allows, in many cases, the establishment of a definitive diagnosis, the redefinition of the prognosis, and the contribution to the appropriate counseling of couples (Chapter 3 and 6).
In cases of chromosomal abnormalities, autopsy provides additional information to the morphological characterization of chromosomal abnormalities. It may also improve the knowledge of the evolving phenotype and natural history, at early gestational ages (Chapter 4 and 5).

In the particular case of Down syndrome, a better knowledge of the fetal phenotype may help to define new ultrasound markers that could improve prenatal identification (Chapter 5).
An autopsy with full evaluation of the heart is important for the detection and better characterization of cardiac anomalies (Chapter 4 and 6).

Capítulo 1

Introdução

1.1 Aspectos históricos

Anomalias congénitas

As anomalias congénitas são defeitos estruturais ou funcionais presentes ou determinados ao nascimento. Consideram-se *major* as anomalias que têm significado clínico ou social, e *minor* aquelas que não têm importância médica nem estética. As anomalias congénitas podem ter causas cromossómicas, monogénicas, multifactoriais (envolvendo factores genéticos e ambientais), teratogénicas ou, na maioria dos casos, ter causa desconhecida.

As anomalias congénitas têm sido representadas por todo o Mundo desde há longa data. A representação mais antiga foi descoberta no sudeste da Turquia, data de 6500 AC, e trata-se de uma estátua de mármore de uma deusa gémea bicéfala[1]. Relatos escritos de anomalias congénitas chegam-nos da Babilónia, na forma de tabuletas de argila encontradas nas escavações da Biblioteca Real de Nínive. São descritas sessenta e duas anomalias agrupadas em cinco grupos que incluem as orelhas, a cabeça, os membros superiores, os intestinos e o aparelho reprodutor e os membros inferiores. Acredita-se que esses relatos datam de 4000 AC[1].

Hipócrates (460-370 AC), considerado o pai da medicina ocidental, baseou a sua prática médica na observação e no estudo do corpo humano. Rejeitou superstições, lendas e crenças, que consideravam que a causa das doenças eram forças superiores. Considerou que as doenças eram o produto de factores ambientais, da dieta e de estilos de vida[1].

Ambroise Paré (1510-1590) elaborou uma classificação e explicação de "monstros" no século XVI (*Des Monstres et Prodiges*, 1585) e William Harvey (1578-1657) na sua obra *Generatione Animalium* em 1651, descreve a malformação chamada "lábio leporino", que considera ser devida a uma paragem no desenvolvimento[1].

Síndrome de Down

A síndrome de Down (DS [MIM #190685]) foi descrita pela primeira vez em termos clínicos em 1866 pelo Dr. John Langdon Down (1828-1896) no artigo *Observations on an Ethnic Classification of Idiots*[2]. Langdon Down era o responsável médico do *Royal Earlswood Asylum for Idiots* em Redhill, Surrey no ano de 1856. Classificou um grupo de pacientes com atraso mental com base nas características étnicas e descreveu um grupo especial *Mongolian idiot.* "*It is to this division I wish, in this paper to draw special attention. The very large number of congenital idiots are typical mongols. So marked is this that when placed side by side it is difficult to believe that the specimens compared are not children of the same parents. The number of idiots who have arranged themselves around the Mongolian type is so great and they present such a close resemblance to one another in mental power that I shall describe an idiot member of this racial division selected from the large number who have fallen under my observation."* A descrição da síndrome em maior detalhe refere: *"The face is flat and broad and destitute of prominence. The cheeks are roundish and extended laterally. The eyes are obliquely placed and the internal canthi more than normally distanced from one another. The palpebral fissure is very narrow. The tongue is long, thick and much roughened. The nose is small. The skin has a slight dirty yellowish texture and is deficient in elasticity, giving the impression of being too large for the body.*"[2].

Antes da descrição clínica efectuada por Down, outros autores tinham referido características fenotípicas da síndrome de Down. Em 1838, Jean-Étienne Dominique Esquirol (1772-1840), um psiquiatra da escola de Pinel, interessado nas diferenças fenotípicas entre o atraso mental e a psicose, descreveu fenotipicamente a síndrome de Down. Em 1846, o francês Édouard Onésimus Séguin (1812-1880), publicou um tratado sobre a "a educação dos idiotas" no qual estabeleceu o primeiro programa de treino para crianças com atraso mental e deu uma descrição da síndrome de Down, a que chamou "*idiotie furfuracée*"[3, 4]. Ainda mais antigas são as cerâmicas e pinturas representando indivíduos com a síndrome de Down. Em 2005, Martinez-Frias publicou uma das evidências históricas mais antigas da síndrome de Down. É uma estátua em terra-cota realizada em meados do século I (cerca do ano 500), que pertence à cultura Tolteca do México. São facilmente identificados aspectos que definem a face de um indivíduo com síndrome de Down: as fissuras palpebrais curtas, os olhos oblíquos, a hipoplasia da face media e a boca aberta com macroglossia[5]. Em 2006, Bernal e Briceno publicaram uma avaliação de artefactos provenientes da cultura Tumaco-La Tolita existente entre as actuais Colômbia e Equador há aproximadamente 2500 anos. Em várias cerâmicas observaram características sugestivas de síndrome de Down[6].

Diferentes autores referem pinturas com representações de indivíduos com síndrome de Down entre os séculos XV e XVI[7, 8]. Levitas e Reid dedicaram especial atenção a uma pintura do século XVI com uma representações de um anjo com características de síndrome de Down[8]. Trata-se de uma pintura, óleo sobre madeira, datada de 1515, intitulada "*The Adoration of the Christ Child*" atribuída por vários especialistas a um artista da escola flamenga, que se encontra no *Metropolitan Museum of Art* em Nova Iorque[8].

Em 1932, muito antes da descoberta do cariótipo humano, Waardenburg (um oftalmogista holandês) e Davenport (um geneticista americano) afirmaram que a síndrome de Down era provavelmente causada por uma anomalia cromossómica[3, 4, 9]. Em 1959, Lejeune e colaboradores[10] e Jacobs e colaboradores[11] descobriram que a trissomia do cromossoma 21 era a causa da síndrome de Down.

A síndrome de Down é causada pela presença em triplicado (trissomia) de todo ou da porção crítica do cromossoma 21. Na maioria dos casos a anomalia cromossómica responsável pela síndrome de Down é a trissomia 21 (95%), sendo as translocações robertsonianas responsáveis por 4% e o mosaicismo por 1% dos casos[12]. O cromossoma 21 extra tem origem materna em 95% dos casos e origem paterna em apenas 5%[13]. A síndrome de Down deve-se à não disjunção meiótica em 95% dos casos, o que origina que um dos gâmetas seja dissómico para o cromossoma 21. A não disjunção meiótica ocorre em 90% dos casos na gametogénese materna e em apenas 10% dos casos na espermatogénese. Nas situações com origem materna a não disjunção ocorre maioritariamente na meiose I (80% dos casos), enquanto nas situações com origem paterna a não disjunção ocorre sobretudo na meiose II (80% dos casos)[14]. A não disjunção meiótica da gametogénese materna está associada a uma idade materna mais avançada, quer ocorra na primeira quer na segunda divisão, mas a não disjunção meiótica na espermatogénese não está associada à idade. A não disjunção meiótica está também relacionada com uma diminuição da recombinação genética[15].

O cromossoma 21, com cerca de 300 genes, é o cromossoma humano mais pequeno. Nenhum gene se associa especificamente ao fenótipo da trissomia 21, sabendo-se que para que exista síndrome de Down é necessário apenas estar em triplicado uma pequena porção do cromossoma 21, a chamada região crítica, composta por vários genes localizados em vários loci[16]. Em 1996 foi identificada a região crítica, uma região com 1,6 Mb entre LA68 e ERG, localizada em 21q22[17]. O cromossoma 21 é um cromossoma

pobre em genes ("gene-poor") com uma razão elevada de sequências AT/GC. A pobreza relativa deste cromossoma explica porque alterações numéricas ou estruturais têm consequências menos graves e importantes do que alterações em cromossomas mais ricos em genes, como os cromossomas 13 e 18.

Anomalias cardíacas

O primeiro relato conhecido de uma anomalia cardíaca remonta a 4000 AC, uma ectopia cordis, que aparece nas tabuletas de argila encontradas nas escavações da Biblioteca Real de Nínive[18]. Em 1513 Leonardo da Vinci desenha e descreve um caso de defeito do septo auricular[19].

É contudo no século XVII que surge o interesse pelo conhecimento das anomalias cardíacas, decorrente de estudos necrópsicos. Em 1671 Niels Stensen descreveu o primeiro caso da anomalia cardíaca, actualmente conhecida como tetralogia de Fallot, devido à descrição efectuada em 1888 por Etienne-Louis Arthur Fallot[20-23]. Em 1679 Theophile Bonet relata o caso de morte súbita num homem com estenose aórtica provavelmente congénita[20, 24].

O primeiro tratado importante sobre cardiologia foi publicado por Jean-Baptiste Sénac em 1749, *Traité de la structure du coeur, de son action, et de ses maladies*[20, 25]. Com base na dissecção anatómica e estudos necrópsicos, escreveu um tratado sobre fisiologia e anatomia cardíacas, antes do desenvolvimento de técnicas de diagnóstico como a auscultação cardíaca[25]. Neste tratado há referências ao defeito do septo interventricular[20]. Em 1814 John Richard Farré publicou *Pathological Researches. Essay I: On Malformations of the Human Heart*, o primeiro tratado dedicado às anomalias cardíacas congénitas, baseado na correlação clinico-patológica[20, 26]. Farré descreve defeitos septais auriculares e ventriculares, atrésia pulmonar, transposição das grandes artérias, estenoses valvulares arteriais e atrioventriculares[20, 26].

John Paget em 1831 escreve *An Inaugural Dissertation On Congenital Malformations of the Heart*, referindo-se à embriologia, possíveis etiologias, histórias clínicas e patologia. Elabora um sistema de classificação com três grupos: *malformations of defect, malformations of excess,* e *malformations of position*[26]. Em 1858 Thomas Peacock publicou um tratado dedicado às anomalias cardíacas congénitas *On Malformations of the Human Heart with Original Cases*, no qual propõe um novo sistema de classificação: *congenital misplacements of the heart*, *deficiency of the pericardium, malformations of the heart* e *malformations in primary development of the vessels*. Neste livro é dado relevo à correlação clinico-patológica e são apresentadas ilustrações detalhadas[26].

Em 1866 Wilhelm Ebstein descreve a anomalia da válvula tricúspide que tem actualmente o seu nome[20].

John M. Keating e William A. Edwards em 1888 publicam *Diseases of the Heart and Circulation in Infancy*, no qual incluem um esquema da circulação fetal[26].

O conhecimento das anomalias cardíacas foi aumentando ao longo dos anos, tornando possível o aparecimento da terapêutica cirúrgica. Em 1939 é relatado o encerramento do canal arterial patente[27] e em 1945 a construção do shunt Blalock-Taussig[28].

Nos anos 60 e 70 do século passado, o desenvolvimento da ecocardiografia e da angiografia cardíacas tornaram possível a descrição *in vivo* das anomalias cardíacas congénitas [29].

Cerca de 12% das anomalias cardíacas são devidas a anomalias cromossómicas[30], quer aneuploidias quer microdelecções (del22q11.2), mas a maioria não tem causa identificável. São factores de risco associados aos defeitos cardíacos a diabetes, infecções e ingestão de fármacos[31]. Algumas são devidas a hereditariedade multifactorial, estando a importância dos factores genéticos a aumentar, com a identificação de genes específicos e de regiões cromossómicas[32-36].

1.2 Aspectos epidemiológicos

Prevalência das anomalias congénitas

As anomalias congénitas *major* afectam 2-3% dos recém-nascidos[37-39] e as anomalias *minor* estão presentes em 14,7-35,8% dos recém-nascidos[40, 41]. Devido à letalidade inerente a muitas das anomalias congénitas, a prevalência das anomalias é maior em embriões e fetos do que em recém-nascidos, pelo que os estudos que também incluem abortamentos e mortes fetais apresentam uma prevalência superior de anomalias ao nascimento (6,9%)[41].

A prevalência das anomalias congénitas aumenta com a idade devido às doenças de aparecimento ou diagnóstico tardio, estimando-se que aos 25 anos 7,9% dos indivíduos tenham uma anomalia congénita[42].

Devido à história familiar[43, 44], patologia associada[45, 46] ou exposição a agentes teratogénicos[47-49], algumas mulheres estão em risco de ter um feto com anomalias do desenvolvimento[50]. Contudo, a maioria ocorre em grávidas sem factores de risco previamente identificados.

As anomalias congénitas em recém-nascidos pré-termo são mais do dobro do que em recém-nascidos de termo[51]. A prevalência de anomalias congénitas é também superior na gravidez múltipla, sendo mais elevada na gravidez monocoriónica do que na bicoriónica[52].

Estimativas da prevalência de aneuploidias em gâmetas referem valores de 18-19% em oócitos e 3-4% em espermatozóides[53]. A prevalência de anomalias cromossómicas no início da gravidez estima-se em 8%[54]. Nos casos de perdas embrio-fetais a prevalência de anomalias cromossómicas é de 60% nos abortamentos precoces e de 6% nas mortes perinatais[54].

A síndrome de Down é a anomalia cromossómica mais frequente a atingir a viabilidade, com uma prevalência ao nascimento de 1,6 a 1,8 por 1000[55-60]. Durante a gravidez a prevalência diminui com a idade gestacional e aumenta com a idade materna. Por exemplo, uma mulher de 35 anos tem às 10 semanas um risco de 1 em 229, às 16 semanas um risco de 1 em 280 e ao nascimento, um risco de 1 em 356[61].

As anomalias cardíacas congénitas são o defeito estrutural mais comum nos recém-nascidos, com uma prevalência ao nascimento de 3,3 a 8,0 por 1000[30, 62-66]. Se forem incluídos todos os tipos de anomalias, como defeitos musculares do septo interventricular ou válvula aórtica bicúspide, a incidência é de 75 por 1000 nados-vivos[67].

Morbilidade e mortalidade associada às anomalias congénitas

Algumas anomalias são letais, condicionando abortamento espontâneo (perda de vitalidade desde a concepção até às 24 semanas), morte fetal (perda de vitalidade ocorrida desde as 24 semanas até ao nascimento) ou morte neonatal precoce (morte que ocorre desde o nascimento até aos 7 dias de vida pós-natal).

Cerca de 50% das concepções humanas são perdidas, muitas delas antes de serem clinicamente reconhecidas[68]. A incidência na população geral de abortamentos ocorridos em gravidezes previamente diagnosticadas é de cerca de 12%[69]. A maioria das perda ocorre durante o período embrionário (até às 10 semanas de gestação)[70], sendo em muito menor quantidade as ocorridas durante o período fetal (após as 10 semanas de gestação) e diminuindo ao longo da gravidez.

As alterações citogenéticas contribuem para metade das perdas precoces[71-74], com 86% de alterações numéricas, 6% de alterações estruturais e 8% de outras alterações cromossómicas[73].

Outras causas genéticas de abortamento foram identificadas[73], nomeadamente doenças monogénicas[75], doenças multifactoriais[76], inactivação selectiva do cromossoma X[77], mosaicismo confinado à placenta[78] e fenómenos de *imprinting*[79].

As anomalias congénitas contribuem substancialmente para a mortalidade em todos os grupos etários. Em muitos países desenvolvidos, com o declínio da taxa de mortalidade infantil, as malformações congénitas representam agora a causa mais frequente de mortalidade durante o primeiro ano de vida. Nos EUA, a taxa de mortalidade global decresceu de 47,0 por 1000 em 1947 para 6,7 por 1000 nados-vivos em 2006[80]. As malformações congénitas tornaram-se a principal causa de mortalidade infantil em 1997, com 22,1% de todas as mortes infantis, em comparação com 15,1% em 1970[81]. Actualmente continuam a constituir a principal causa de morte infantil, com 20,1% das mortes infantis em 2006[80]. No Reino Unido a mortalidade perinatal foi de 4,2 por 1000 em 2008[82], sendo as anomalias congénitas responsáveis por 8,3% das mortes fetais e por 20,0% das mortes neonatais[82]. Em Portugal, a taxa de mortalidade perinatal foi de 4,6 por 1000 em 2009[83]. Em 2006, as anomalias congénitas contribuíram para 10,3% das mortes perinatais (período perinatal alargado)[84].

A taxa de mortalidade infantil específica por anomalias cromossómicas é 22,4 por 100.000 nados-vivos e as trissomias dos autossomas contribuem para mais de 80% das mortes infantis neste grupo[81]. A taxa de mortalidade infantil devida a síndrome de Down nos EUA, foi 2,3 por 100.000 em 1985-1987 e 2,5 por 100.000 nados-vivos em 1995-1997[81]. As principais causas de morte em indivíduos com síndrome de Down são as anomalias cardíacas e as infecções respiratória [85, 86].

O aparelho cardiovascular é responsável pela mais elevada taxa de mortalidade nos Estados Unidos, com uma taxa de mortalidade específica de 1,27 por 1000 nados-vivos em 1970-1976 e 0,63 por 1000 em 1991-1997 [81]. De acordo com um relatório recente, 45% e 37% das mortes infantis devidas a anomalias congénitas foram causadas por defeitos cardíacos, na Europa Ocidental e nos EUA, respectivamente[87].

As anomalias congénitas e particularmente as anomalias cardíacas continuam a ser uma importante causa de morte ao longo da vida. Os jovens com anomalias congénitas têm um risco de morte 13 vezes superior aos jovens sem cardiopatia[88].

Para além da mortalidade que condicionam, os defeitos congénitos contribuem substancialmente para a morbilidade infantil e ao longo da vida.

A síndrome de Down é a principal causa de atraso mental de etiologia genética identificada. Para além disso, está associada a múltiplas anomalias estruturais e co-morbilidades, como infecções respiratórias, leucemia e demência precoce[89, 90], que condicionam uma necessidade aumentada de cuidados de saúde e de suporte social[91].

As anomalias cardíacas congénitas são responsáveis por inúmeras hospitalizações, exigem uma equipa multidisciplinar diferenciada e tecnologia avançada de diagnóstico e terapêutica, necessitam de suporte social específico e acarretam uma diminuição da qualidade de vida dos doentes e das suas famílias[92, 93].

Ao longo dos anos tem-se verificado um aumento da sobrevida dos indivíduos com anomalias congénitas, nomeadamente síndrome de Down e anomalias cardíacas[86, 90, 94]. A melhoria da sobrevida deve-se à melhoria dos cuidados perinatais e, no caso específico das anomalias congénitas, ao avanço das técnicas cirúrgicas[95].

1.3 Evolução do rastreio e diagnóstico pré-natais

Rastreio pré-natal

Durante os últimos anos, extensa investigação identificou vários marcadores bioquímicos e ecográficos associados a síndrome de Down, no primeiro e no segundo trimestres de gravidez. Em 1984 foi encontrada uma associação entre alfa-fetoproteína baixa e síndrome de Down[96]. Desde essa altura, outros marcadores bioquímicos foram associados a risco aumentado para síndrome de Down: hormona gonadotrofina coriónica (hCG)[97], estriol não conjugado (uE3)[98], inibina-A[99, 100], fracção livre da β-hCG[101] e proteína plasmática associada à gravidez (PAPP-A)[102]. A translucência da nuca, referida pela primeira vez por Nicolaides e colaboradores em 1992[103], é o marcador ecográfico de cromossomopatia que, isoladamente, tem maior taxa de detecção[104]. Posteriormente, outros marcadores ecográficos do primeiro trimestre foram definidos, como o ducto venoso[105-107], os ossos do nariz[108, 109] ou a regurgitação tricúspide[110, 111]. Mais recentemente foi definido um novo marcador, o fluxo na artéria hepática, que poderá vir a revelar-se útil[112, 113].

Vários métodos de rastreio, combinando marcadores ecográficos e/ou bioquímicos, foram sendo propostos com taxas de detecção superiores a 90% para 5% de falsos positivos[114-117].

Diagnóstico pré-natal

As anomalias congénitas podem ser diagnosticadas *in utero*, após o nascimento ou na infância. Algumas têm manifestação tardia pelo que só são diagnosticadas na idade adulta.

Diagnóstico morfológico

O diagnóstico pré-natal de anomalias morfológicas é efectuado com recurso a técnicas de imagem, destacando-se pelo seu papel preponderante a ecografia.

As primeiras utilizações da ecografia na clínica devem-se a Ian Donald, que em 1957 obtém a primeira imagem ecográfica de um feto e em 1958 publica no *Lancet* o artigo *"Investigation of abdominal masses by pulsed ultrasound",* considerado um dos mais importantes na história da ecografia[118-120]. No entanto, só em 1984 o *Royal College of Obstetricians and Gynecologists* recomendou o seu uso por rotina na prática clínica.

O primeiro relato do diagnóstico pré-natal por ecografia de uma anomalia congénita refere um caso de hidrocefalia efectuada por Donald em 1961, num dos seus primeiros artigos sobre ecografia[121]. Em 1964 é publicado um caso de diagnóstico de acrania efectuado no terceiro trimestre[122].

O desenvolvimento da ecografia fez com que a sua utilização se tornasse universal, constituindo um pilar do diagnóstico pré-natal. Tem aplicação quer como método de rastreio (avaliação de marcadores de cromossomopatias) quer como método de diagnóstico (diagnóstico de anomalias major).

A ecografia como método de diagnóstico

A ecografia no 1º trimestre possibilita o diagnóstico de algumas anomalias *major*[123-125], mas é no 2º trimestre que tem maior acuidade de diagnóstico, pelo que quando realizada entre as 20 e as 22 semanas é chamada de "ecografia morfológica". Algumas anomalias de tradução tardia, nomeadamente do sistema nervoso central, digestivo ou urinário, só são possíveis de diagnosticar no 3º trimestre de gravidez[126].

A acuidade diagnóstica depende da idade gestacional, do número de exames efectuados, da experiência do operador, da qualidade do aparelho, das características da população e do tipo e prevalência das anomalias.

A acuidade diagnóstica varia entre 13,3-81,8%, em estudos realizados entre 1978 e 1997[127]. A acuidade foi mínima (13,3%) no estudo de Ashe e colaboradores, realizado na Irlanda entre 1985 e 1986, envolvendo 6869 fetos[128], e máxima (81,8%) no trabalho de Campbell e Pearce, realizado em Inglaterra entre 1978 e 1982, com 9526 fetos[129]. No estudo RADIUS realizado nos EUA, com uma prevalência de anomalias de 2,3% ao nascimento, o diagnóstico pré-natal foi de 34,8%, com apenas 16,6% de anomalias diagnosticadas antes das 24 semanas[130, 131]. O estudo Eurofetus, prospectivo e multicêntrico (60 centros europeus, dos quais nove portugueses), englobando 4615 malformações em 3686 fetos, encontrou uma taxa de detecção de fetos com malformações de 61,4% e uma taxa de detecção de malformações de 56,2%. A sensibilidade para o diagnóstico de malformações *major* foi de 73,7% (contra 45,7% para as anomalias *minor*), com uma grande variabilidade entre orgãos, com sensibilidade máxima para o sistema nervoso central (88,3%) e sistema urinário (84,8%) e mínima para o coração e grandes vasos (38,8%)[132, 133]. Num estudo realizado em França, o diagnóstico de anomalias isoladas foi de 26,2% e de anomalias múltiplas de 66%[134]. A acuidade diagnóstica é mais elevada no caso de anencefalia (100%) e malformações do tracto urinário (59%) e mais baixa nos casos de fenda labial (17,5%) e defeitos de redução dos membros (18,2%)[134]. Num estudo realizado em Oxford[38], entre 1991 e 1996, com uma prevalência de 2,2% de anomalias *major*, tiveram diagnóstico pré-natal 55% dos casos. O diagnóstico pré-natal aumentou de 42% nos três primeiros anos do estudo para 68% nos últimos três. A acuidade do diagnóstico variou com o tipo de anomalia, com o diagnóstico de pé boto isolado em apenas 28% das situações, contra 98% de diagnóstico nos casos de defeito tubo neural[38]. O EUROCAT, um estudo populacional englobando 17 registos europeus, nos anos de 1995 a 1999, incluía 4366 recém-nascidos com malformações (11 malformações graves), dos quais 64% tiveram diagnóstico pré-natal[135]. Num outro estudo realizado numa população de baixo risco (82% exames eram rotina), realizado entre 1984 e 1999 na Suécia, com uma prevalência de malformações de 2,6%, o diagnóstico pré-natal foi efectuado em 28,4% dos casos, com 49% detectadas antes das 23 semanas[136]. No mesmo período, um estudo realizado na Noruega, entre 1989 e 1999, com uma prevalência de 1,5% de anomalias, relatou uma taxa de detecção de 38,7% na ecografia do 2º trimestre[137].

Diagnóstico de cardiopatias

A avaliação pré-natal do coração e o diagnóstico de anomalias cardíacas iniciou-se nos anos 70 do século passado. Em 1980 foi publicada uma correlação ecocardiográfica e anatómica, efectuada com o objectivo de definir os planos a utilizar na avaliação cardíaca fetal[138]. Nesse mesmo ano, é publicado um artigo na qual se descrevem várias anomalias cardíacas (estruturais e alterações de ritmo) diagnosticadas *in utero*[139].

O diagnóstico pré-natal de defeitos cardíacos é difícil, com taxas de detecção em populações de baixo risco entre 13,7 e 57%[63, 133, 140-144]. A taxa de detecção é inferior em idades gestacionais mais precoces[145] e superior quando é efectuado um ecocardiograma por um cardiologista pediátrico especializado[146].

As grávidas com factores de risco para anomalias cardíacas (história familiar, diabetes mellitus, exposição a teratogénicos, anomalia extra-cardíaca), são habitualmente referenciadas para efectuar ecocardiograma fetal, apesar de esta estratégia de rastreio permitir apenas detectar 10% dos fetos afectados[147]. A

possibilidade de diagnóstico de anomalias cardíacas aumenta com a avaliação da translucência da nuca[148-153], do fluxo no ducto venoso[106, 154-157] e do fluxo na válvula tricúspide[158] no primeiro trimestre da gravidez. Estes métodos de rastreio seleccionam um grupo de grávidas com risco aumentado, que beneficiarão da realização posterior de ecocardiograma fetal[159].

Técnicas invasivas e diagnóstico citogenético e molecular

Há referência à utilização de técnicas invasivas desde há mais de 100 anos, mas foi nos anos 50 e 60 do século passado que a amniocentese foi descrita na abordagem da doença hemolítica fetal[160-162], tornando-se um procedimento de rotina na Obstetrícia.

As primeiras referências à determinação do sexo fetal no líquido amniótico datam de 1956[163]. Esta técnica possibilitou a interrupção de gravidez em casos de doenças monogénicas ligadas ao cromossoma X, como a hemofilia A, quando o feto era do sexo masculino[164, 165].

Em 1966 Steele e Breg demonstraram a possibilidade de efectuar cariótipo fetal a partir de células obtidas por amniocentese[166] e em 1968 surge uma das primeiras referências ao diagnóstico pré-natal da síndrome de Down[167]. Nadler e Gerbie publicaram em 1970 no *New England Journal of Medicine* um importante artigo, "*Role of amniocentesis in the intra-uterine diagnosis of genetic defects"*, que foi impulsionador das técnicas invasivas diagnósticas[168].

Nos anos 70 do século passado a amniocentese passou a ser efectuada sob controlo ecográfico[169].

As técnicas invasivas acarretam um risco de abortamento de 0,5% a 2%[170-174], pelo que estão reservadas a grávidas com risco aumentado de ter um feto afectado.

Diagnóstico de síndrome de Down

Actualmente o diagnóstico pré-natal de anomalias cromossómicas só pode ser efectuado em células fetais obtidas por procedimentos invasivos, como a amniocentese, a biópsia das vilosidades coriónicas ou a cordocentese.

Tradicionalmente o diagnóstico efectua-se através da realização do cariótipo, uma técnica eficaz, mas laboriosa e demorada. Alternativamente, pode ser efectuado mais rápida e economicamente, pela detecção de estruturas genómicas da região crítica, pelas novas técnicas de biologia molecular ou de citogenética, como o FISH (*Fluorescent In-Situ Hybridization)*[175, 176], QF-PCR (Q*uantitative Fluorescent Polymerase Chain Reaction)*[177, 178], MLPA (*Multiplex Ligation-dependent Probe Amplification*)[179, 180]. Apesar de controverso, mais recentemente têm sido utilizados os array-CGH (*Array Comparative Genomic Hybridization*) para diagnóstico pré-natal de aneuploidias[181, 182].

Estão em desenvolvimento técnicas de diagnóstico não invasivo que poderão num futuro próximo permitir o diagnóstico de síndrome de Down através da identificação de DNA fetal livre em circulação materna. Várias metodologias têm sido desenvolvidas[91, 183-186], mas as mais promissoras são actualmente as que se baseiam na identificação de DMRs (*differentially methylated regions*) através da imunoprecipitação do DNA metilado (MeDiP)[187].

1.4 Actuação perinatal

Ao detectar anomalias graves, o diagnóstico pré-natal possibilita a optimização dos cuidados perinatais, com intervenções *in utero* ou com o nascimento programado em centros diferenciados.
O diagnóstico pré-natal permite aos casais preparação e adaptação para o nascimento de um filho com uma anomalia congénita[188-190].

Tratamento intra-uterino
O tratamento fetal iniciou-se em 1963, quando Liley efectuou a primeira transfusão intra-uterina num caso de anemia por aloimunização anti-D[191]. Desde então várias terapêuticas fetais têm sido utilizadas[192, 193], como os anti-arrítmicos no tratamento das disritmias [194, 195] ou a fotocoagulação laser no síndrome de transfusão feto-fetal[196]. Noutras situações, como as válvulas da uretra posterior[197, 198], a hérnia diafragmática congénita[199] ou o mielomeningocelo[200], as intervenções intra-uterinas melhoraram o prognóstico.
Estão em desenvolvimento intervenções *in utero* em situações de anomalias cardíacas, nomeadamente estenose valvular aórtica ou pulmonar[201-204], com o objectivo de melhor o prognóstico dessas patologias.

Programação do parto
A programação do nascimento em centros diferenciados permite optimizar e melhorar os resultados em várias patologias, como a hérnia diafragmática[205], as massas cervicais[206] ou a patologia cardíaca[207]. A diminuição da mortalidade nas anomalias cardíacas, condicionada pelo diagnóstico pré-natal, apesar de não ser consensual[208, 209], parece ocorrer em algumas situações[210-213]. Excepto para as formas mais graves de anomalias cardíacas, o diagnóstico pré-natal melhora o prognóstico, imediato e a longo prazo, ao possibilitar a programação do parto e da actuação neonatal. São melhores, especificamente, os resultados neurológicos das crianças submetidas a intervenção cirúrgica[208, 214, 215].

Interrupção médica da gravidez
O diagnóstico pré-natal possibilita ainda a opção pela interrupção médica da gravidez, nos casos de malformação grave ou letal[216-220].
O primeiro caso de interrupção médica da gravidez na sequência do diagnóstico pré-natal de anencefalia foi publicado em 1972[221].
A decisão pela interrupção de gravidez é complexa e pode ser influenciada por vários factores, como a escolaridade, os antecedentes obstétricos, a idade gestacional, o tipo e gravidade da anomalia ou a existência de anomalias associadas[216, 220]. Factores psicológicos interferem também com a decisão e com o modo como essa decisão é percepcionada[222]. A gravidade das anomalias é um determinante importante para a decisão dos casais[217, 223, 224]. O tipo de anomalias interfere também com a decisão, sendo as anomalias do sistema nervoso central as que têm maior proporção de interrupção[135, 224]. Nas aneuploidias o prognóstico é dos factores determinantes mais importantes[218, 219], desempenhando as anomalias ecográficas associadas um papel preponderante na decisão do casal[225-228].
O recurso à interrupção médica da gravidez veio condicionar uma diminuição da mortalidade perinatal por malformações congénitas[229-231].
O diagnóstico pré-natal e a interrupção médica da gravidez condicionam uma alteração na prevalência das anomalias ao nascimento. Apesar do aumento da idade das grávidas observa-se uma diminuição de

nascimentos de crianças com síndrome de Down[12]. Também a prevalência e tipo de anomalias cardíacas presente ao nascimento se alterou em consequência da interrupção de formas graves[232, 233].

1.5 Autópsia perinatal

No século III AC, em Alexandria, realizaram-se as primeiras autópsias, com aprovação oficial, quer para determinar a anatomia normal quer as alterações condicionadas pelas doenças. As primeiras autópsias foram realizadas com objectivos de ensino e reportam-se a Herophilus (c 335-280 AC)[234]. Um século mais tarde, Erasistratus (c 310-250 AC) efectuava dissecções para observar alterações devidas a doenças[234]. Posteriormente, na Idade Média, há relatos episódicos de realização de autópsias.

Os primeiros textos medico-legais com referências à autópsia encontram-se num tratado chinês do século XIII, *Hsi Yuan Lu* (Instruções para médicos legistas)[235]. Em Itália, na mesma época, são efectuadas autópsias sobretudo com fins médico-legais. No século XV, o Papa Sixtus IV (1471-1484) autoriza a realização de dissecções do corpo humano aos estudantes de Bolonha e Pádua, com confirmação posterior de Clemente VII (1523-1534)[234, 235]. Um novo avanço no interesse pela anatomia é dado por Andreas Vesalius, no livro *Fabrica Humani Corporis*, publicado em 1543[235].

Em 1724 Hermann Boerhaave (1668-1738) descreve o primeiro diagnóstico *post-mortem* de ruptura do esófago[234].

Morgagni (1682-1772), em Pádua, defende a importância da correlação entre os sintomas clínicos e os achados anatómicos, esperando que isso pudesse levar à compreensão da relação entre a função alterada e a estrutura anormal[234], o que constitui a base da patologia. François Xavier Bichat (1771-1802) considerado o Pai da Histologia, correlacionou as doenças com as alterações nos tecidos[234].

François Xavier Bichat, Richard Bright (1789-1858) e Sir William Osler representam uma escola de clínicos que efectuavam autópsias, para esclarecer os processos clínicos que observavam nos seus pacientes, definindo novas doenças[235].

O século XIX é marcado pelos conflitos entre Karl Rokitansky (1804-1878) e Rudolf Virchow (1821-1902). Karl Rokitansky, que efectuou mais de 30000 autópsias, dedicou-se apenas à macroscopia, sendo considerado talvez o maior patologista macroscópico da história. Por seu lado, Rudolf Virchow (1821-1902) dedicou-se à microscopia e contribuiu significantemente para o desenvolvimento das bases teóricas da patologia[234].

A autópsia perinatal, no entanto, deve o seu desenvolvimento a Edith Louise Potter (1901-1993), que desde os anos 30 do século passado dedicou todo o seu trabalho a esta área da patologia, e em 1952 publicou o livro que constitui uma referência na patologia perinatal (*Pathology of the fetus and newborn. Chicago: Year Book Publishers; 1952*)[236]. Nos anos 80 surgem outros trabalhos referindo a importância de realizar exame necrópsico nos casos de morte fetal e neonatal. Barson e Wigglesworth, nos seus livros, referem a importância da patologia perinatal como subespecialidade em simultâneo com o desenvolvimento das especialidades clínicas (Perinatologia) e sobretudo da ecografia.

Em 1988 o *Royal College of Obstetricians and Gynecologists* conjuntamente com o *Royal College of Pathology*, defendem que idealmente a taxa de autósias perinatais deve ser 100%, considerando como mínimo necessário 75%[237]. Em 2002 foi publicado o relatório de um grupo de trabalho cujo objectivo foi desenvolver a patologia fetal, perinatal e pediátrica, contando com as principais sociedades científicas inglesas da especialidade, e que reforça as indicações e recomendações sobre a realização da autópsia perinatal[238].

O papel da autópsia perinatal vai para além de estabelecer ou confirmar a causa de morte. Pode acrescentar informação, não previamente conhecida, relevante para o diagnóstico[239-243], o que ocorre entre 22 a 76% das situações[244]. Estas informações são importantes para o aconselhamento dos casais, podendo alterar o risco de recorrência futura[243, 245].

A autópsia, ao melhorar o conhecimento sobre as doenças e as causas de morte, melhora a informação disponível para a realização de estudos epidemiológicos, contribuindo também para a definição de estratégias de prevenção[246].

Por exemplo, no casos das anomalias cardíacas, a autópsia permite o diagnóstico em casos de crianças que morreram em consequência dessas anomalias e não tinham diagnóstico prévio [247]. Se estes dados não forem tidos em conta nos estudos epidemiológicos, a verdadeira prevalência das anomalias cardíacas não é conhecida. Para além disso, a autópsia pode pormenorizar a descrição anatómica das anomalias[248] e detectar anomalias associadas que alteram o diagnóstico final[249, 250].

A informação fornecida pela autópsia permite aos familiares compreender o que ocorreu, contribuindo para a sua tranquilização[246, 251, 252].

Outra função importante da autópsia é a auditoria, reconhecida nos casos de morte perinatal[239, 240] e em situações específicas, como a patologia cardíaca[248].

A autópsia tem desempenhado ao longo dos tempos um papel importante no ensino pré e pós-graduado[239, 253]. É útil na investigação, permitindo conhecer novas doenças, a sua história natural e o efeito das várias terapêuticas[254].

A realização de autópsias clínicas tem diminuído em todo o mundo, mas as autópsias perinatais têm-se mantido mais elevadas, apesar de também estas terem começado a decair[243, 255-258]. As taxas de autópsia perinatal variam entre 16 a 100% (média de 38%), com taxas de 5-100% nas mortes fetais, 33-100% nas mortes neonatais e 79-100% nas interrupções de gravidez[244].

A maior limitação à realização da autópsia perinatal é a ausência de consentimento por parte dos pais[258, 259]. Também a falta de motivação dos clínicos desempenha um papel importante, quer por considerarem que há métodos de diagnóstico alternativos[241, 260], quer por considerarem que a autópsia de que dispõem não tem a qualidade exigida[241, 261]. Cerca 25-50% das autópsias perinatais não têm os critérios mínimos de qualidade definidos[241, 261-263].

1.6. Avaliação da qualidade

A preocupação com a qualidade dos cuidados de saúde prestados tem vindo a motivar não só os profissionais de saúde como toda a sociedade, o que levou ao desenvolvimento de vários programas de avaliação da qualidade dos cuidados de saúde. O diagnóstico pré-natal e a interrupção médica da gravidez são das actividades em que essa avaliação é mais importante.

Existe alguma confusão com o significado dos termos "garantia de qualidade" e "controlo de qualidade", frequentemente usados com o mesmo significado, apesar de serem diferentes. Segundo a *American Society of Quality* a garantia de qualidade refere-se às actividades planeadas e implementadas com o objectivo de assegurar que os requisitos de qualidade sejam mantidos, enquanto o controlo de qualidade consiste em técnicas de avaliação usadas para assegurar os requisitos de qualidade[264].

Várias sociedades científicas (ACOG, RCOG, ISUOG) têm definido regras para a realização de ecografia obstétrica que contribuem para a garantia de qualidade. Deve efectuar-se um registo correcto dos resultados dos exames, o arquivo de relatórios e imagens e uma correlação com os desfechos clínicos[265-267]. Indispensáveis para a garantia de qualidade são também os processos de acreditação e de certificação. A acreditação é para os Serviços, sendo importante que o equipamento seja de qualidade, esteja a funcionar correctamente, sejam cumpridas as regras éticas e de segurança, haja arquivo de relatórios e imagens, sejam cumpridas as regras definidas pelas entidades superiores e os profissionais sejam qualificados[268]. A certificação, que garante a qualificação dos profissionais, é um processo individual[268].
A maioria dos programas iniciais de avaliação da qualidade da ecografia baseavam-se na detecção ou não de anomalias[127, 269-271]. Como a prevalência de anomalias é baixa, estes programas de avaliação de qualidade baseados na detecção de anomalias são pouco abrangentes. Têm, entretanto, sido desenvolvidos outros métodos de avaliação de qualidade com base em parâmetros objectivos que devem ser avaliados em todas as ecografias, de modo a que todos os que efectuem este exame possam ser sujeitos a avaliação[272]. A avaliação da translucência da nuca foi o primeiro parâmetro ecográfico a ser submetido a controlo de qualidade[273-275]. Desde então, vários métodos de avaliação de outros parâmetros, como o comprimento crânio-caudal, perímetro abdominal, perímetro cefálico ou comprimento do fémur, têm sido elaborados[276-278]. À semelhança do que aconteceu com a avaliação da translucência da nuca, os métodos de avaliação da qualidade a utilizar devem ser qualitativos e quantitativos[272].
Um outro processo de efectuar a avaliação de qualidade do diagnóstico pré-natal é a comparação entre o diagnóstico pré-natal e o diagnóstico *post-mortem*. Há no entanto poucos trabalhos que correlacionam o diagnóstico pré-natal com o diagnóstico *post-mortem*, sendo na maioria muito heterogéneos, englobando um pequeno número de casos e praticamente todos retrospectivos[241, 258, 279-281].

1.7 Legislação em diagnóstico pré-natal

Os cuidados pré-natais variam de país para país, independentemente da existência ou não de recomendações[282]. Decorrente da variação das recomendações sobre vigilância pré-natal e da legislação, as estratégias de rastreio, diagnóstico pré-natal e interrupção de gravidez são necessariamente diferentes[283]. As leis da interrupção médica de gravidez por motivos fetais, nos países da Europa, variam no limite da idade gestacional considerada. Em alguns países é permitida até ao termo, noutros países é possível até uma determinada idade gestacional e noutros ainda é possível até termo apenas em casos de anomalias letais[283].
Em Portugal, a partir de 1989, foi desenvolvido e implementado o programa de Saúde Materna e Infantil. Os objectivos, os princípios e o modelo de organização das actividades de diagnóstico pré-natal, foram estabelecidos em 1997, no *Despacho 5411/97, de 6 de Agosto (2ª série)*. A respectiva estrutura, os requisitos necessários e as orientações para a sua concretização no terreno, foram estabelecidos em 1999, no *Despacho 10 325/99, de 26 de Maio (2ª série).* A interrupção da gravidez foi despenalizada em 1985 (*artigo 142.º do Código Penal, com as alterações introduzidas pelo Decreto-Lei n.º 48/95, de 15 de Março, e pela Lei nº 16/2007, de 17 de Abril*). Na alínea c) estão definidas as situações fetais em que a interrupção está despenalizada: quando "Houver seguros motivos para prever que o nasciturno virá a sofrer, de forma incurável, de grave doença ou malformação congénita, e for realizada nas primeiras 24

semanas de gravidez, excepcionando-se as situações de fetos inviáveis, caso em que a interrupção poderá ser praticada a todo o tempo". O enquadramento dos motivos de causa fetal é avaliado por uma comissão de três a cinco médicos (obstetras com diferenciação em ecografia, pediatras, geneticistas), como determinado pela *Lei nº 189/98, de 24 de Março, e modificada pela Lei nº 741-Q/2997, de 21 de Junho.*

1.8 Ética em diagnóstico pré-natal

A ética é uma dimensão importante da medicina e em particular da obstetrícia, nomeadamente da medicina fetal.

A decisão da grávida de efectuar rastreio ou diagnóstico pré-natal de cromossomopatias deve basear-se no conceito do consentimento informado[284, 285].

A abordagem clínica de uma gravidez complicada por anomalia fetal coloca também questões éticas[286, 287]. Um dos princípios mais importantes da ética médica é a obrigação de proteger e respeitar os interesses do paciente. Na obstetrícia deparamo-nos com a particularidade da existência de dois pacientes. Em relação à mulher grávida os princípios da beneficiência e da autonomia devem ser respeitados. Em relação ao feto, o princípio da autonomia não se aplica, pois o feto não possui crenças nem valores. Já o princípio da beneficiência, aplica-se quando se considera o feto como paciente, o que implica que o feto seja viável, ou seja, que tem possibilidade de existência *ex utero*[286]. Tem sido questionado se após a viabilidade fetal a interrupção médica da gravidez colide com o princípio da beneficiência. Chervenak e colaboradores consideram que perante uma alta probabilidade de morte ou de comprometimento do desenvolvimento neurológico a obrigação de respeitar o princípio da beneficiência não é violado[287-289].

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Capítulo 2

Objectivos

Objectivos

O objectivo principal deste trabalho é a correlação entre o diagnóstico pré-natal e o *diagnóstico post-mortem* com vista à melhoria da acuidade do diagnóstico pré-natal. Os objectivos específicos são:

1. Correlação entre o diagnóstico pré-natal e o diagnóstico *post-mortem* nas situações de interrupção médica da gravidez de causa fetal (Capítulo 3)

2. Contributo da autópsia nos casos de interrupção médica da gravidez por anomalias cromossómicas (Capítulo 4)

3. Variabilidade fenotípica na síndrome de Down (Capítulo 5)

4. O contributo da autópsia perinatal nas anomalias cardíacas congénitas (Capítulo 6)

Capítulo 3

Correlação entre o diagnóstico pré-natal e o diagnóstico *post-mortem* nas situações de interrupção médica da gravidez de causa fetal

3.1 Critical evaluation of elective termination of pregnancy in a tertiary fetal medicine centre during 43 months: correlation of prenatal diagnosis findings and postmortem examination

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Critical evaluation of elective termination of pregnancy in a tertiary fetal medicine center during 43 months: correlation of prenatal diagnosis findings and postmortem examination

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Objectives The aim of this study is the critical evaluation of cases of elective termination of pregnancy (TOP) in the Prenatal Diagnosis Center of S. João Hospital.

Material and Methods We performed a retrospective study of cases of elective TOP over a 43-month period in a tertiary referral hospital. The fetal indications for termination were analyzed. A comparative study of the prenatal diagnosis, established by ultrasound, and the results of postmortem findings, was performed. These were classified as having complete agreement, complete disagreement and major agreement with additional information.

Results In total, during this period, 76 elective terminations of pregnancy were performed due to fetal causes. The number of fetal identified grounds was 25 cases of chromosomal abnormalities, 36 cases of morphological anomalies and 15 cases of other fetal situations. The comparison between ultrasound and fetopathologic findings showed complete agreement of diagnosis in 61.1% of cases, and no case of absolute discordance was identified. Major concordance with additional information was found in 38.9% of cases, with an increased risk of recurrence in six cases, and a decreased estimated risk of recurrence in three cases.

Discussion This study reinforces the importance of the systematic evaluation of all cases of elective TOP by autopsy performed by a specialist fetal pathologist. This is the most reliable way of assessing the adequacy of prenatal diagnosis and implementing quality control. More than corroborating or correcting the prenatal diagnosis, systematic autopsy may establish a definite diagnosis, adjust prognosis and may be helpful in counseling the parents for a future pregnancy. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: termination of pregnancy; prenatal diagnosis; audit; postmortem examination/autopsy

INTRODUCTION

Since 1995, the Portuguese law (law dated 15th March 1995, no. 142 of DL no. 48/95 and later modified in 30th July 1997, no. 90/97) authorizes legal termination of pregnancy (TOP) in three distinct circumstances: (1) TOP is authorized until 12 weeks for maternal reasons, when continuing pregnancy is undoubtedly hazardous for a woman's physical or mental health; (2) TOP is allowed until 24 weeks for fetal reasons, when a fetus has a high risk of presenting incurable or highly handicapping diseases; or at any time of pregnancy, if the fetus presents an inviable condition; (3) TOP is allowed until 16 weeks when the pregnancy results from sexual assault against a woman's will.

In such situations, the ascertainment of maternal circumstances is corroborated by a medical report. The ascertainment of fetal circumstances is evaluated by a commission of three or five practitioners (obstetricians specialised in ultrasound; pediatricians, geneticists), as determined by law no. 189/98 dated 24th March 1998.

The world-wide increase in the number of elective terminations of pregnancy prompted the critical evaluation/audit of the prenatal findings compared to postmortem information, in order to warrant quality control, confirmation of diagnosis and adequate parental counseling. Thus, the aim of the present study is to apply this critical evaluation to our own results of a 43-month period of TOP in a tertiary referral center for fetal medicine. Further, it was our aim to evaluate the potential benefit of objective postmortem examination in enhancing accurate diagnosis and adequate counseling.

MATERIAL AND METHODS

A retrospective evaluation of the cases of elective TOP for fetal causes that occurred between January 2002 and August 2005 in the Prenatal Diagnosis Center of S. João Hospital (a tertiary and teaching hospital) was performed. This evaluation was based on searching clinical records extensively. It included evaluation of medical reports justifying the termination, the medical indications, the legal adequacy and the time interval

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for completing the process. The maternal age and the gestational age at the time of TOP were also considered for assessment.

The fetal indications for TOP were divided into three types: morphological anomalies, chromosomal abnormalities and other causes.

Comparison of the degree of concordance or discordance between the prenatal findings and the postmortem information was the key question. As a result of this comparison, the cases were classified as having complete agreement, complete disagreement and major agreement with either minor or significant additional information (Porter and Keeling, 1987). Significant additional findings were those that would have affected the final diagnosis, and eventually affect the clinical genetics counseling, but would not have altered the management of pregnancy. Minor additional findings have no clinical relevance and do not expectantly change management.

RESULTS

During this 43-month period, 77 requests for TOP were submitted to the evaluation commission. One request was denied, since it did not match with the legal dispositions (one Klinefelter syndrome without any associated malformation). Spontaneous fetal death occurred in one case.

In the 76 TOPs owing to fetal causes, the proportion of the indications was as such: 25 cases of chromosomal abnormalities, 36 of morphological anomalies and 15 cases of other causes (Table 1).

The detection of chromosomal abnormalities by invasive testing was motivated by increased nuchal translucency in eight cases, positive biochemical screening in seven cases, positive integrated screening test in one case, and morphologic anomalies detected in ultrasound in five cases. In five cases, the reason for performing amniocentesis was unavailable. Maternal age was not an indication in any case. The chromosomopathies included 17 cases of trisomy 21, two cases of trisomy 13, two cases of trisomy 18, two cases of trisomy 9, one case of monosomy X and one case of deletion of the short arm of chromosome 18. In 14 of the chromosomal anomalies, morphological alterations were detected: in all cases of trisomy 9, trisomy 13 and monosomy X, in eight out of 17 cases of trisomy 21 and in one out two cases of trisomy 18.

The morphological anomalies are summarized in Table 2: central nervous system anomalies (21), cardiac defects (3), renal anomalies (4), abdominal wall defects (5), multiple anomalies (1) and other anomalies (thoracopagus conjoined twins, sacrococcygeal teratoma).

Table 1—Fetal indications for elective termination of pregnancy

Chromosomal abnormalities		25
Morphological anomalies		36
Others fetal causes		15
Fetal infection	2	
Premature rupture of membranes, anhydramnios	9	
Monogenic disease	1	
Environmental or toxic exposure	3	
Total		76

Table 2—Morphological anomalies (chromosomal excluded) that determined elective TOP

Central nervous system anomalies		21
Acrania	9	
Arnold-Chiari anomaly	7	
Ventriculomegaly	4	
Microcephaly	1	
Cardiac anomalies		3
Tricuspid atresia with right ventricle hypoplasia	1	
AV septal defect, complete AV block, fetal hydrops	1	
Double discordancy AV and VA and pulmonary atresia	1	
Renal anomalies		4
Urethral obstruction syndrome	2	
Bilateral multicystic kidneys	1	
Renal dysplasia, anhydramnios	1	
Abdominal wall defects		5
Gastroschisis	1	
Omphalocele	4	
Multiple anomalies		1
Others		2
Thoracopagus conjoined twins	1	
Sacrococcygeal teratoma	1	
Total		36

The other fetal causes (Table 1) were grouped under fetal infections (two cases of cytomegalovirus (CMV) infection), premature rupture of membranes before 24 weeks (nine cases), monogenic disease (one case of Steinert type 1 distrophy, 1000 triplets CTG) and environmental and toxic exposure (three cases of exposure to teratogenic drugs—misoprostol, chemotherapy and isotretinoin).

In all cases, the postmortem examination was performed. When comparing the prenatal and postmortem findings in the 72 cases of fetal cause with morphological anomalies or chromosomal abnormalities (excluding the monogenic disease and the toxic exposure), complete agreement was found in 44 cases (61,1%) (Table 3), and major agreement with additional findings in 28 cases (38,9%) (Table 4). In no case was total disagreement identified.

In the cases of total agreement (Table 3), 18 chromosomopathies are included, ten anomalies of the central nervous system (nine of the neural tube), eight cases of preterm membrane rupture before 24 weeks, and two cases of CMV infection.

Major agreement with significant additional findings at postmortem was described in 28 cases (Table 4). Within this group, we would like to enlighten a case of acrania caused by amniotic bands, a case of renal tubular dysgenesis and two cases of omphalocele with other anomalies. In those cases in which the postmortem examination did not conform to all the ultrasound findings (trisomy 21 suspected of having a cardiac defect), the indication for TOP was not altered.

Taking into consideration the additional information at postmortem, there was a correction for the increased risk of recurrence in six cases (renal tubular dysgenesis,

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Table 3—Cases of complete agreement between prenatal findings and postmortem findings

Prenatal findings	Number of cases	Postmortem findings
Chromosomal abnormalities		
Trisomy 21, cardiac defect	2	Characteristic anomalies, cardiac defect
Trisomy 21	9	Characteristic anomalies
Trisomy 13, holoprosencephaly	2	Characteristic anomalies (holoprosencephaly)
Trisomy 18, hydrops, growth restriction, limb anomalies	1	Characteristic anomalies (hydrops, growth restriction, limb anomalies)
Trisomy 18	1	Characteristic anomalies
Trisomy 9	2	Characteristic anomalies
Monosomy X, cystic hygroma	1	Characteristic anomalies (cystic hygroma)
Morphologic anomalies and others fetal causes		
Acrania	5	Acrania
Arnold-Chiari anomaly	4	Arnold-Chiari anomaly
Ventriculomegaly	1	Ventriculomegaly
Tricuspid atresia with right ventricle hypoplasia	1	Tricuspid atresia with right ventricle hypoplasia
Urethral obstruction syndrome	2	Urethral obstruction syndrome
Huge omphalocele	1	Huge omphalocele
Thoracopagus conjoined twins	1	Thoracopagus conjoined twins
Sacrococcygeal teratoma	1	Sacrococcygeal teratoma
Cytomegalovirus (CMV) infection, microcephaly	1	CMV viral inclusion in brain, kidneys and placenta
Cytomegalovirus infection, hepatic calcifications, hyperechogenic bowel	1	CMV viral inclusion in liver, lungs, pancreas, kidneys and placenta
PROM <24 weeks with anhydramnios	8	Acute chorioamnionitis, bilateral pneumonia (1), swallowing of infected amniotic fluid (1), pulmonary hypoplasia (1)

Table 4—Cases of major agreement with additional information at postmortem examination

	Postmortem findings	
Prenatal findings	Confirmatory	Additional
Chromosomal abnormalities		
del (18) (p11.2)	Characteristic anomalies	Complex cardiac defect
Trisomy 21, polimalformed fetus	Characteristic anomalies	Without cardiac defect
Trisomy 21, cardiac defect (4)	Characteristic anomalies	Without cardiac defect
Trisomy 21, cystic hygroma	Characteristic anomalies	Without cystic hygroma
Morphologic anomalies and others fetal causes		
Hydranencephaly	Hydranencephaly	Unilateral renal agenesia, hypoplasia pulmonary
Hydrocephaly with macrocephaly	Hydrocephaly with macrocephaly	Posthemorragic hydrocephaly
Hydrocephaly with macrocephaly	Hydrocephaly with macrocephaly	Hydrocephaly by lymphocitic meningitis of infectious origin
Arnold-Chiari anomaly, mielomeningocele	Arnold-Chiari anomaly, mielomeningocele	Minor anomalies. Terminal deletion of long arm of chromosome X
Arnold-Chiari anomaly, mielomeningocele	Arnold-Chiari anomaly, mielomeningocele	Cardiac defect, unilateral renal dysplasia
Arnold-Chiari anomaly, mielomeningocele, hydranencephaly	Arnold-Chiari anomaly, mielomeningocele	Severe hydrocephaly, *corpus calosum* agenesis
Microcephaly	Microcephaly	Arnold-Chiari anomaly type II without cerebellar herniation
Acrania	Acrania	Anoftalmia, facial deformities. Deletion of chromosome 18
Acrania	Acrania	Anomalies compatible with chromosomopathies. Trisomy 18

(*continued overleaf*)

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Table 4—(*Continued*)

Prenatal findings	Postmortem findings	
	Confirmatory	Additional
Acrania	Acrania	Amniotic bands syndrome
Encephalocele	—	Acrania, minor anomalies. Deletion of chromosome 18
AV septal defect, complete AV block, fetal hydrops	AV septal defect, fetal hydrops	Noncompacted myocardium
Double discordancy AV and VA and pulmonary atresia	Double discordancy AV and VA and pulmonary atresia	Situs inversus
Bilateral multicystic kidneys	Multicystic dysplastic kidneys	Minor anomalies
Renal dysplasia	—	Renal tubular dysgenesis
Gastroschisis, megacystis	—	Body stalk anomaly
Huge omphalocele	Huge omphalocele	Complex cardiac defect
Huge omphalocele	Huge omphalocele	Holoprosencephaly, minor anomalies
Huge omphalocele	—	Body stalk anomaly
Polimalformed fetus (cystic hygroma, abdominal wall defect, lumbar hyperlordosis)	Omphalocele	Defect of segmentation of spinal column. Monosomy X
PROM, anhydramnios		Noncompacted myocardium

noncompacted myocardium) and decreased risk of recurrence in three cases (acrania by amniotic bands, hydrocephaly by lymphocytic meningitis of infectious origin, and polimalformed fetus that turned out be a monosomy X).

The median maternal age of the women submitted to TOP was 31 years, varying from 16 to 42 years. We observed an increase in the mean maternal age at pregnancy for TOP motivated by chromosomal abnormalities when compared to other groups (34.0 *vs* 30.4 years, $p = 0.0090$). Noteworthy was the fact that in the group of chromosomal anomalies, 46% of pregnant women were 35 years or more.

Overall, the gestational age at the time of TOP varied between 7 and 35 weeks, with a mean of 18 weeks. Six TOPs were performed before 12 weeks, 29 between 13 and 16 weeks, and 36 between 17 and 24 weeks. The six TOPs performed after 24 weeks were preceded by feticide.

The mean time that elapsed between the day of the request and the beginning of the TOP process was 3,8 days (0–40 days). The case that lasted 40 days was a dichorionic twin pregnancy with discordance of malformation.

DISCUSSION

Our study presents data on elective TOP, assessing the degree of concordance and discordance between prenatal findings and postmortem information.

The morphological anomalies constitute the most frequent indication for TOP (47%), followed by chromosomal abnormalities (33%) (39% *vs* 35% in the French study of Guillem *et al*., 2003).

A trained perinatal pathologist subjected all TOP cases to postmortem examination. Unfortunately, this is not always the case in others institutions: in a retrospective study performed in Oxford, between 1991 and 2000, 309 cases of TOP for structural anomalies were evaluated. In what concerns the rate of cases studied by autopsy, it decreased from 84% in the period 1991–1995 to 67% in 1996–2000 (Boyd *et al*., 2004). In another retrospective study performed in Birmingham, in which the ultrasonographic prenatal diagnosis was compared with postmortem findings in 153 cases of fetal death, the postmortem examination was performed only in 30.7% of the cases (Johns *et al*., 2004).

In the present study, the comparison between prenatal findings and postmortem data yielded a total agreement in 61.1% of the cases, and no case of total discordance was identified. These figures were similar to those found in other studies, with rates of agreement of about 40–60%. In an above-mentioned article, Boyd *et al*. found a total concordance in 55% of cases and a total discordance in 2% (Boyd *et al*., 2004). In the study published by Johns *et al*., a total concordance was obtained in 46.8% of the cases, and a total discordance in 2% (Johns *et al*., 2004). In a retrospective study performed in Hungary between 1992 and 1998, in which 173 cases of abortions were evaluated (121 were TOP), a total agreement was found in 48.8% of the cases and a total discordance in 2.5% (Kaiser *et al*., 2000).

The postmortem examination revealed to be of utmost importance to parental counseling, since it enhanced the confirmation, revision or extension of the prenatal diagnosis. In our study, the autopsy redefined the risk of recurrence in 9/28 (32%), very similar to the one found by Boyd (27%) (Boyd *et al*., 2004).

Our data confirm the accuracy of prenatal diagnosis in differentiated centers, while emphasizing the importance of postmortem examination in establishing the definitive diagnosis and redefining prognosis. Autopsy is the keystone of quality control for prenatal diagnosis, teaching and research.

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The TOP performed after 24 weeks of pregnancy represented only 8.3% of the cases, a rate significantly lower than in others countries, namely in France, where the TOP after 24 weeks attained 32.9% (Guillem *et al*., 2003). This significant difference is justified by a distinct TOP policy in France that allows termination for nonlethal fetal anomalies throughout pregnancy.

This study reinforces the importance of strict and critical evaluation of TOP cases to warrant quality control of the prenatal diagnosis proposed. Not only does postmortem examination refine the initial diagnosis, but it also clarifies the prognosis and contributes to the adequacy of parental counseling for future pregnancies.

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3.2 Revisiting acrania: same phenotype, different etiologies

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Revisiting Acrania: Same Phenotype, Different Aetiologies

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Key Words
Acrania · Amniotic band syndrome · Anencephaly · Autopsy · Cytogenetics · Foetal ultrasound · Genetic counselling · Prenatal diagnosis · Termination of pregnancy

Abstract
Objective: To evaluate the contribution of prenatal and postmortem examinations in establishing the aetiology of acrania. ***Methods:*** Retrospective evaluation of 14 cases of acrania managed through elective termination of pregnancy. ***Results:*** The median maternal age was 30 years (range 18–40) and median gestational age at diagnosis was 13 weeks (range 12–15). One mother had epilepsy and was taking anticonvulsants and another had uncontrolled type II diabetes mellitus. Only 3 women were using folic acid at conception. Chromosomal abnormalities were detected in 3 of 8 cases analyzed. Unilateral anopthalmia, cervical rachischisis, midline facial and limb defects coexisted with acrania in 4 cases. Acrania with craniofacial dysmorphism and asymmetrical finger amputation were observed in a case of amniotic band syndrome. A previous history of anencephaly was documented in 1 case. ***Conclusion:*** Acrania is a characteristic phenotypic expression of a variety of different aetiologies. Investigation with cytogenetic studies and postmortem are essential to provide a definitive answer. This will provide a better understanding of the underlying aetiology and help establish the recurrence risk for future pregnancies.

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Introduction

Acrania is a rare congenital anomaly characterized by absence of the flat bones of the cranial vault exposing the neural tissue, which subsequently degenerates (exencephaly/anencephaly). A diagnosis of acrania can be made by ultrasound after 11 weeks' gestation, by which time ossification of the skull normally occurs.

The evolution from acrania to exencephaly and finally anencephaly is largely accepted [1–4]. According to the acrania-exencephaly-anencephaly sequence hypothesis, cerebral tissue not protected by meninges, cranium and skin is progressively destroyed in utero due to exposure to amniotic fluid and mechanical trauma. This combination of intrauterine insults produces the classic anen-

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Fig. 1. B-Mode and Colour Doppler Ultrasound scan at 12 weeks gestation showing an absent cranial vault and exposed neural tissue (case 14).



Fig. 2. Anencephaly-meroacrania, unilateral anophtalmy and median cleft lip in a foetus subsequently found to have partial deletion of chromosome 18 (case 2).

cephalic features with flattened brain remnants behind prominent orbits in the second and third trimesters.

The prevalence of acrania varies considerably according to the population being studied, geographical location, time and selected maternal demographic characteristics. Variations may be due to true heterogeneity and socio-economic differences among populations, differences in surveillance methodology and registries, and the impact of prenatal diagnosis and elective termination for affected pregnancies. In an American population, foetuses of Caucasian and Hispanic ethnicity were found to be more frequently affected than foetuses of African descent, and female foetuses are more frequently affected than males [5]. In Portugal the reported prevalence was 3.06/10,000 births between 1997 and 1999 [6].

Acrania typically results from a multi-factorial process involving a number of developmental genes and other environmental factors. Whilst our understanding of the specific genes involved in neural tube defects (NTDs) remains incomplete, genes involved in folate metabolism are believed to be important. Mutations in 1 such gene, methylenetetrahydrofolate reductase (MTHFR), have been shown to be associated with an increased risk of NTDs. A second gene, VANGL1, a membrane-associated signalling complex protein has also been shown to be associated with an increased risk of NTDs [7]. Environmental risk factors for NTDs include hyperthermia, uncontrolled pregestational diabetes, anti-epileptic drugs, exposure to lead, maternal obesity and folate deficiency [5]. NTDs are also known to be associated with chromosomal abnormality, other single-gene defects and disruption of the amniotic membrane. Whilst the association between NTDs and a variety of chromosomal abnormalities is well reported, few series have looked at acrania alone. We report our experience in attempting to define the underlying aetiology in 14 foetuses with a prenatal diagnosis of acrania in the first or early second trimester of pregnancy.

Materials and Methods

We present a retrospective cohort of foetuses found to have acrania at the Prenatal Diagnosis Centre of a tertiary hospital during a 6-year period (January 2002–December 2008). Ultrasound scans were performed transabdominally and/or transvaginally (Aloka5000, Aloka, Japan, or Voluson730, GE, USA). The sonographic diagnosis (fig. 1) was established based on the following criteria: absence of the cranial vault (acrania); exposure of neural tissue with an irregular echogenicity (exencephaly) or absent neural tissue (anencephaly); bulging foetal orbits ('frog-like' appearance).

After discussion of the prognosis for the pregnancy, all parents decided to interrupt the pregnancy and medical termination using misoprostol was performed in all cases in order to allow foetal autopsy. Postmortem examinations were performed according to standardized procedures and with parental consent. The postmortem pathologic criteria for exencephaly included absence of the scalp and calvarium (acrania) with the cerebral mass protected by a thick 'dura mater' like membrane; cerebral tissue consisting of a disorganized, anarchic growth of nervous tissue with polymicrogyria and nodules of heterotopias, or more frequently showing degenerative change. Anencephaly (fig. 2) was diagnosed when the cranial vault was congenitally absent (acrania) with ce-

Table 1. Clinical, sonographic, pathologic and cytogenetic findings in a series of 14 foetuses with acrania defined at 12–15 weeks gestation

Case	Age years	GA[1] weeks	Sonographic findings	Pathologic findings	Genetic findings	Other history
1	34	13	Acrania	Anencephaly-meroacrania	–	No folic acid
2	35	14	Acrania	Anencephaly-meroacrania, unilateral anophtalmy, median cleft lip and cleft palate	Deletion 18	No folic acid
3	31	13	Exencephaly	Anencephaly-meroacrania	–	Post-conceptional folic acid
4	26	13	Acrania	Anencephaly-meroacrania	–	No folic acid
5	32	15	Anencephaly	Anencephaly-meroacrania	–	Post-conceptional folic acid
6	30	13	Acrania	Anencephaly-meroacrania, bilateral absence of thumb, halux hypoplasia and a cleft between halux and second finger in both feet	Deletion 18	Peri-conceptional folic acid
7	40	12	Exencephaly	Foetus lost for study; anomalous chorionic villi	Trisomy 18	Post-conceptional folic acid
8	18	12	Acrania	Anencephaly-meroacrania	–	Epilepsy and carbamazepine; post-conceptional folic acid
9	39	13	DC twin pregnancy: (1) Acrania (2) Blighted ovum	(1) Amniotic bands with meroacrania, severe facial dismorphy, fingers amputations (2) Nine-week embryo; no anomalies	–	Post-ICSI; peri-conceptional folic acid
10	28	13	Acrania	Anencephaly-meroacrania, unilateral anophtalmy	46,XY	No folic acid
11	24	12	Exencephaly	Anencephaly-meroacrania	46,XX	Previous anencephaly (22 weeks); no folic acid
12	25	14	Exencephaly	Anencephaly-meroacrania	46,XX	Uncontrolled diabetes type II; peri-conceptional folic acid
13	30	12	Exencephaly	Anencephaly-holoacrania and cervical rachischisis	46,XX	Post-conceptional folic acid
14	25	12	Exencephaly	Anencephaly-meroacrania	46,XY	No folic acid

DC = Dichorionic; ICSI = Intracytoplasmic sperm injection. [1] Gestational age at termination (weeks).

rebral hemispheres completely missing or decreased to small masses attached to the base of the skull. The malformation was further subclassified in meroacrania when the defect did not involve the foramen magnum, holoacrania when the defect extended to the foramen magnum, and holoacrania with rachischisis when spina bifida accompanied anencephaly [8].

In the earlier part of this study karyotyping by multiplex ligation-dependent probe amplification (MLPA) after DNA extraction from paraffin blocks was only performed in cases in which further anomalies were detected. More recently, MLPA has been performed routinely in all cases of medical termination of pregnancy.

Results

Fourteen affected foetuses were recognised in the first or early second trimester. Their clinical, sonographic, pathologic and cytogenetic characteristics are summarized in table 1. The median maternal age was 30 (range 18–40) years and the median gestational age at diagnosis was 13 (range 12–15) weeks. Two of the 14 women had chronic medical conditions, 1 being affected by epilepsy and taking anti-convulsivant therapy, the other presenting with uncontrolled type II diabetes mellitus. One woman had a history of a previous pregnancy affected by anencephaly. Only 3 women were using folic acid at the time of conception, 5 initiated it after knowing they were pregnant and 6 took no folic acid supplementation at all. One pregnancy was a dichorionic twin (resulting from intracytoplasmic sperm injection) – the second twin being a blighted ovum.

Isolated acrania, confirmed by necropsy, was present in 8 cases. In 5 cases the necropsy added further information (table 1): facial anomalies in 2 (cases 2 and 10), anomalies of the extremities in 1 (case 6) and cervical rachischisis in another (case 13). One foetus (case 9), had severe craniofacial dysmorphism in addition to acrania, together with asymmetric finger amputations suggestive of amniotic band syndrome. In 1 case (No. 7) pathological examination was restricted to the placenta, which revealed anomalous chorionic villi suggestive of a chromosomal abnormality. Cytogenetic evaluation was performed in 8 cases and revealed chromosomal abnormalities in 3: partial deletion of chromosome 18 in 2 cases (1 and 6) and trisomy 18 (case 7) in the third.

Discussion

Acrania is a developmental anomaly that can be defined by ultrasound at the end of the first trimester of pregnancy, once the embryological process of cranial ossification is complete. In this series, the sonographic diagnosis of acrania ranged between 12 and 15 weeks' gestation, demonstrating the feasibility of accurate diagnosis of the malformation by targeted examination of the foetal head and brain at an early gestational age. Likewise, the sonographic and pathologic evidence presented here support the acrania-exencephaly-anencephaly sequence theory.

The vast majority of cases of isolated acrania are multifactorial, implicating a number of genes that play a role in the development of the neural tube that interact with environmental agents. In this series, 2 of 8 cases of isolated acrania had a defined environmental factor: namely uncontrolled maternal type II diabetes mellitus in 1 case and epilepsy with anti-convulsivant medication in the second. Although periconceptional supplementation of maternal diet with folate is recognised to reduce the prevalence and recurrence of neural tube defects by more than 50% [9], only a minority of women (3 of 14) had complied with established international guidelines. Folate supplementation at the minimum required dose (0.4 mg/day) to all women of childbearing age and/or a national food fortification policy would probably be cost-effective public health care measures in our population. A higher dose (4 mg/day) is recommended for prevention of recurrence and for epileptic women and this dose should be maintained throughout pregnancy in the latter group.

Acrania can also occur as part of a malformation syndrome resulting from known chromosomal abnormalities (trisomies 18, 13 and 21, triploidy, mosaic trisomy 11 and 20, and a number of deletions and duplications) or single gene disorders (i.e. Meckel Gruber syndrome) [10–12]. 3 of 8 (38%) of the foetuses karyotyped in this series were found to have a chromosomal abnormality; higher than the rate reported in the literature (0.66–5.56%) [13–17]. This difference may be due to the small number of cases but also to the early gestational age of diagnosis of the malformations reported in this series. It is important to note, however, that simple fragment deletions found by MLPA should ideally be confirmed by an independent method and this is a potential flaw in our study. The issue of routine chromosomal analysis for prenatally diagnosed cases of NTDs is controversial. We would suggest that defining a chromosomal abnormality is important: whilst the recurrence risk for an unexplained NTD is 2–4% and for an autosomal recessive genetic condition is 25%, the risk of recurrence of trisomy 18 is typically much lower.

Whilst acrania is very obvious on prenatal ultrasound, other structural anomalies may be more subtle, and a foetal autopsy is important in the process of establishing whether there is evidence of an underlying chromosomal abnormality or genetic syndrome. Parental karyotyping may also be indicated to establish recurrence risks.

One foetus in this series showed evidence of being affected by an amniotic band. This is an unusual, but well recognised cause of acrania, where disruption of the amnion results in fibrous bands entangling various foetal parts in utero – causing single or multiple anomalies. The prevalence of amniotic bands has been estimated at 1/1,200 to 1/15,000 live births [18] – although higher in 1 series of spontaneous abortions (1 in 56 pregnancies) [19]; the male-to-female ratio is 1:1. A search for amniotic bands and a detailed survey of the foetal face, digits and body must be included in the sonographic evaluation of foetal acrania in order to rule out this syndrome. This is believed to be a sporadic disorder with no increased risk in a subsequent pregnancy, except in rare familial cases of epidermolysis bullosa and Ehlers-Danlos syndrome [20].

To conclude, although acrania-exencephaly-anencephaly share a similar and characteristic phenotype, different aetiologies can often be found. Postmortem pathologic and cytogenetic studies are essential to provide a definite diagnosis, to improve our understanding of the aetiologies that underlie this malformation and to establish recurrence risks for future pregnancies.

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3.3 Renal tubular dysgenesis: report of two cases in a non-consanguineous couple and review of the literature

Ramalho C, Matias A, Brandão O, Montenegro N. Renal tubular dysgenesis: report of two cases in a non-consanguineous couple and review of the literature. Fetal Diagn Ther 2007; 22: 10–13

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Renal Tubular Dysgenesis: Report of Two Cases in a Non-Consanguineous Couple and Review of the Literature

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Key Words
Renal tubular dysgenesis · Prenatal diagnosis · Autosomal recessive · Oligohydramnios

Abstract
Renal tubular dysgenesis is a rare congenital abnormality of renal development characterized by short and poorly developed proximal convoluted tubules. It is associated to late-appearing oligohydramnios, Potter's sequence, pulmonary hypoplasia and calvarial bone hypoplasia with enlarged fontanels. The onset of oligohydramnios is delayed and variable, normally later than 22 weeks of gestation, conditioning variation in the expression of the other deformities. We report 2 cases of renal tubular dysgenesis occurring in a non-consanguineous couple. Copyright © 2007 S. Karger AG, Basel

Introduction

Renal tubular dysgenesis is a rare congenital abnormality of renal development [1, 2]. This condition was first described by Allanson et al. [1] in 1983 in two stillborn sibs from a non-consanguineous couple, with diffuse renal tubular hypoplasia. It is a rare cause of complete anuria in the newborn period, which translates in the prenatal period as oligohydramnios, Potter's sequence and pulmonary hypoplasia, leading to death a few hours or days after birth.

We report two cases of renal tubular dysgenesis in which the definitive diagnosis was only accomplished postnatally by autopsy. The prenatal course was similar in both cases, prompting further investigation to clarify the diagnosis with consequent redefinition of prognosis.

Case Report

A 26-year-old pregnant woman, gravida III para II, non-consanguineous couple, was referred to our center at 27 weeks of gestation for intra-uterine growth restriction (IUGR) and oligohydramnios.

The first pregnancy was uneventful, and a healthy girl was born.

The second pregnancy was complicated by IUGR and oligohydramnios detected at 29 weeks. Cesarean section was performed at 32 weeks, with the delivery of a girl with deformities caused by oligohydramnios. She had anuria and died on the first day of life due to respiratory failure. The autopsy confirmed IUGR and pulmonary hypoplasia, and demonstrated multifocal solid renal dysplasia and calvarial bone hypoplasia with enlarged fontanels.

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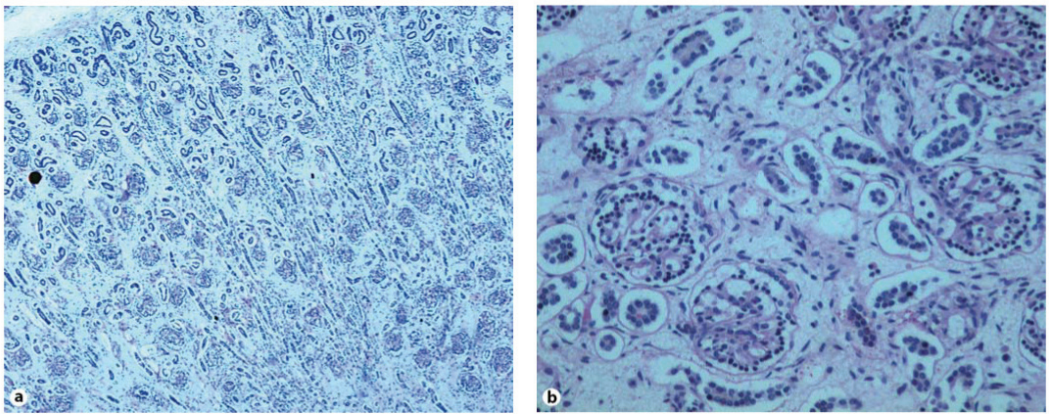


Fig. 1. Kidney with glomerular crowding and immature tubules. The apical surfaces of the proximal tubule cells have not taken up the stain. PAS staining. **a** ×40. **b** ×200.

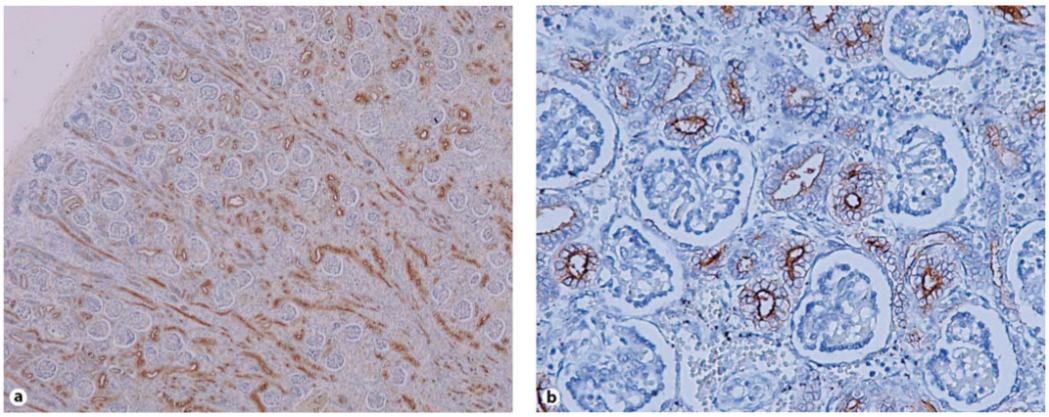


Fig. 2. Kidney with 'primitive' appearance of the tubules. The proximal convoluted tubules show EMA (epithelial membrane antigen) marker. EMA immunohistochemical marker. **a** ×40. **b** ×200.

In the third pregnancy, the ultrasound performed at 22 weeks of gestation revealed no fetal anomalies, including adequate amniotic fluid volume and kidneys of normal size and echogenicity. The pregnant woman had a depression and she was under alprazolam. The ultrasonography at 27 weeks of gestation showed IUGR and oligohydramnios along with normal kidneys and bladder, and with normal flow in the renal arteries. The magnetic resonance imaging confirmed the presence of normal kidneys with two small cysts (3 × 5 mm in diameter).

Relying on the latter ultrasound findings combined with the data of the previous pregnancy, the assumption of renal dysplasia was put forward. The couple was counseled and, in view of the extremely poor prognosis, they decided to terminate the pregnancy. Feticide was performed with KCl 7.5% after fetal anesthesia with thiopental and vecuronium.

Autopsy demonstrated abnormality of renal development, with particular expression in the maturation/differentiation of tubules, compatible with renal tubular dysgenesis. Macroscopic morphol-

ogy of the kidneys and excretory tree was normal. Histology demonstrated alterations in nephrogenesis, particularly in the tubular growth: the number of mature glomeruli was normal and organized; in other areas the glomeruli had a more crowded appearance because of the reduced number of convoluted tubules (particularly proximal tubules); only few immature tubules were identified; mesenchyme was prominent in the corticomedullary transition and in the medullary region. Finally, anomalous cortical vascularization was demonstrated.

The histochemical and immunocytochemical study of the kidneys emphasized the poorly differentiated and reduced number of tubules, namely through the absence of brush borders in the apical surfaces of proximal tubules cells (fig. 1, 2).

All these renal abnormalities coexisted with defects in the ossification of calvarial bone with enlargement of the fontanels.

There was neither evidence of ischemic etiology, namely thrombosis of the renal vein, nor renal anomalies compatible with recessive polycystic autosomal disease or renal cystic dysplasia.

The retrospective histochemical and immunocytochemical evaluation of the deceased second child in the early neonatal period allowed the confirmation of the renal tubular dysgenesis in this case.

Based on clinical, obstetrical, and anatomopathological findings, a diagnosis of renal tubular dysgenesis was made.

The couple was informed that despite the uncertain pathogenesis, the abnormality is inherited as an autosomal-recessive trait, with 25% risk of recurrence. They were also informed about the impossibility of the prenatal diagnosis before the appearance of oligohydramnios, that is, before 25 weeks of gestation.

Discussion

Renal tubular dysgenesis is a rare congenital abnormality of renal development characterized by short and poorly developed proximal convoluted tubules [1–3]. It is associated with late-appearing oligohydramnios, Potter's sequence (distinctive compressed face and limb deformities), pulmonary hypoplasia and calvarial bone hypoplasia with enlarged fontanels. The onset of oligohydramnios is delayed and variable, normally later than 22 weeks of gestation, causing variation in the expression of the others deformities. The pulmonary hypoplasia is not a systematic finding, depending on the duration of the exposure to oligohydramnios [4, 5].

In the postnatal period, the neonate presents anuria and renal insufficiency, with normal appearing kidneys in the ultrasonography.

The renal tubular dysgenesis is inherited as an autosomal-recessive trait, with 25% risk of recurrence in a future pregnancy [2].

The renal tubular dysgenesis is lethal in the neonatal period. The neonates have renal insufficiency and respiratory failure, which causes death in the first 48 h of life.

Prenatal diagnosis of this condition is extremely difficult and practically impossible in the first proband. This clinical condition can only be suspected with the late appearance of oligohydramnios in the presence of normal kidneys in ultrasonography [6].

Patients should be informed about the 25% risk of recurrence (considering the autosomal recessive trait), about the impossibility to make early genetic diagnosis (the mutation is not identified), and that even the clinical suspicion is delayed since it is dependent on the late appearance of oligohydramnios in the presence of normal kidneys at ultrasonography.

The postnatal diagnosis can be suspected in a neonate that presented oligohydramnios antenatally and who shows renal insufficiency of indeterminate etiology, particularly with structurally normal kidneys, after birth.

No genetic forms of renal tubular dysgenesis are associated with fetal exposure to angiotensin-converting enzyme (ACE) inhibitors [7, 8], nonsteroidal anti-inflammatory drugs [9, 10] and twin-twin transfusion syndrome [11, 12]. The phenotypic similarity between the genetic renal tubular dysgenesis and the one associated with ACE inhibition suggests that the molecular pathology in this uncommon autosomal recessive disease could be related to mutations of the gene systems governing the 'renin-angiotensin-aldosterone' system [8]. Recently, Yosypiv and El-Dahr [13] demonstrated that mutations in the genes encoding components of the renin-angiotensin system (RAS) are implicated in the development of the ureteric bud and collecting ducts. Stroma-derived angiotensin II promotes activation of AT1/AT2 receptors expressed on the ureteric bud to stimulate branching morphogenesis as well as collecting duct elongation and papillogenesis. We can, therefore, speculate that a compromise of the RAS may be implicated in the late failure of urine production due to interference with late metanephrogenesis.

The importance of reporting these rarely occurring cases is that only suspicion (and not definitive diagnosis) is possible prenatally, based on late occurring oligohydramnios with normal-appearing kidneys. Genetic confirmation is currently impossible. Definitive diagnosis is always postnatal by the conjugation of clinical and pathological examination [14].

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3.4 Concordance for bilateral congenital diaphragmatic hernia in a monozygotic dichorionic twin pair – first clinical report

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Novel Insights from Clinical Practice

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Concordance for Bilateral Congenital Diaphragmatic Hernia in a Monozygotic Dichorionic Twin Pair – First Clinical Report

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Established Facts

- The concordance for anomalies in both members of a dichorionic twin pregnancy is a very rare event, but it has already been described for congenital diaphragmatic hernia.

Novel Insights

- This is the first report on the occurrence of a bilateral congenital diaphragmatic hernia affecting both fetuses of a monozygotic twin pregnancy.

Key Words
Bilateral congenital diaphragmatic hernia · Monozygotic dichorionic twin pregnancy

Abstract
The authors present a clinical case of concordance for bilateral congenital diaphragmatic hernia in a monozygotic twin pair diagnosed in the early second trimester.

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Introduction

Congenital diaphragmatic hernia (CDH) is a common developmental anomaly with a reported prevalence of one in 2,500–4,000 live births [1].

CDH is mainly a sporadic disease, the incidence of familial cases being difficult to determine [2]. Bilateral diaphragmatic defect is a very rare condition accounting for less than 3% of all CDH [3], but it occurs more frequently in familial cases with a prevalence reaching 10% [4].

We describe a pair of monozygotic twins with concordant bilateral CDH diagnosed in the early second trimester.

Case Report

A 28-year-old healthy pregnant woman, gravida 3, para 2, was referred to our institution with a dichorionic twin pregnancy at 13 weeks' gestation. The couple was nonconsanguineous and the woman's two previous pregnancies were uneventful.

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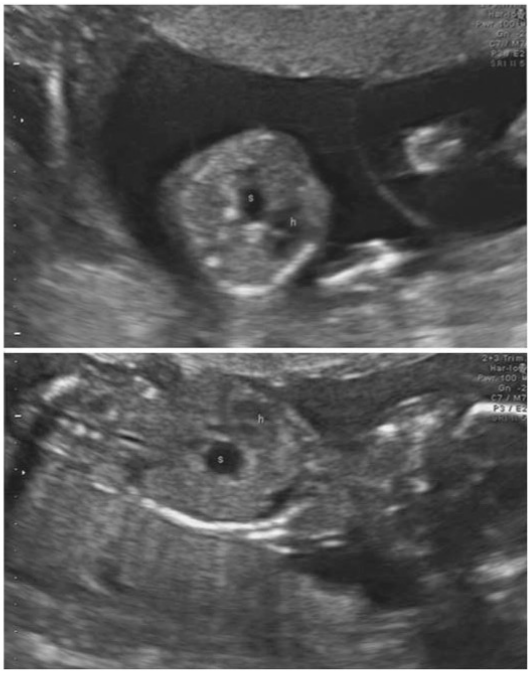


Fig. 1. Ultrasound image of a 16 weeks' gestation fetus showing the stomach in an intrathoracic position at the same level of the heart.

The first trimester ultrasound scan confirmed a dichorionic diamniotic pregnancy showing at 12 weeks one fetus with an increased nuchal translucency (2.7 mm), abnormal A waveform in the ductus venosus and the presence of the stomach in an intrathoracic position raising the suspicion of left CDH. The other fetus presented no anomalies at that time.

The ultrasound scan was repeated at 16 weeks and showed that both fetuses were affected by a left CDH with stomach and bowel loops inside the thorax (fig. 1). There was no significant mediastinum shift. Fetal karyotype was determined by amniocentesis and was normal for both fetuses (46,XX). The couple asked for elective termination of pregnancy at 18 weeks.

The necropsic examination revealed bilateral posterior diaphragmatic defect in both fetuses, more pronounced on the left side (fig. 2, 3). The left pleural space was occupied by the stomach, spleen, bowel and liver left lobe. Liver right lobe was seen in the right pleural space in both fetuses. Lungs were affected by bilateral hypoplasia. The pathological study of both placentas was normal.

Monozygosity was established by DNA analysis.

Discussion

Twin pregnancies are at a higher risk of having at least one malformed fetus, and in these cases the anomalies are often discordant, affecting only one of the siblings, both in dizygotic and monozygotic twins. The concordance for anomalies is a very rare event [5] and it can be interpreted as having a genetic contribution. The occurrence of CDH in both members of a monozygotic twin pair has already been described, but there are no published concordance ratios between monozygotic and dizygotic twins.

The frequency of malformations in dizygotic twins is thought to be similar to that of singletons (2–3%), while it has been reported to be two or three times higher in monozygotic pairs [6]. However, the true incidence is difficult to assess since zygosity is not always evaluated and we are blind to zygosity in 44% of dichorionic same-sex twins.

Structural malformations that are not genetically determined may occur in only one fetus of a monozygotic twin pair, but it has been estimated that in 15% of cases both twins are affected by one structural anomaly [7].

There are several reports on the presence of CDH affecting both fetuses of a twin pregnancy, but to the best of our knowledge this is the first report describing the occurrence of a bilateral anomaly in both twins. The widespread use of antenatal ultrasound examination makes CDH prenatal detection feasible, often in the second trimester of pregnancy. The prenatal diagnosis of bilateral CDH is rare and difficult and can present a diagnostic challenge to the sonographer. As in our report, often the initial diagnosis is a left-sided unilateral anomaly, and the right-sided defect is usually detected during surgical repair or necropsic examination. There are only two reports on a sonographic diagnosis of bilateral CDH made prenatally by the demonstration of the stomach and bowel inside the thorax, absence of mediastinum displacement [8] and by the observation of liver in both thoraces, confirmed by color Doppler of the hepatic vessels [9].

Bilateral CDH occurs more often in familial cases, and it is frequently associated with chromosomal abnormalities and other major anomalies, approaching 70% in the Congenital Diaphragmatic Hernia Study Group series [10]. However, the etiology of CDH remains insufficiently understood either in sporadic or familial situations, raising the suspicion of de novo dominant mutations or epigenetic abnormalities. The frequent finding of associated congenital malformations qualifies CDH not as a lo-

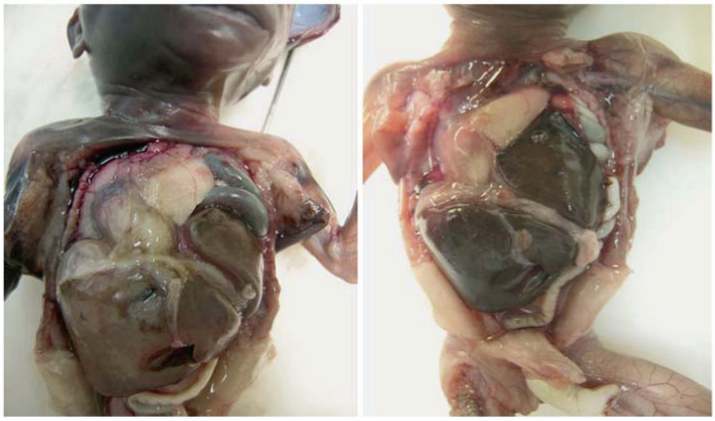


Fig. 2. Necropsic examination of both fetuses with similar features: left hepatic lobe and small bowel loops above the left diaphragm; minor shift of the mediastinum to the right.

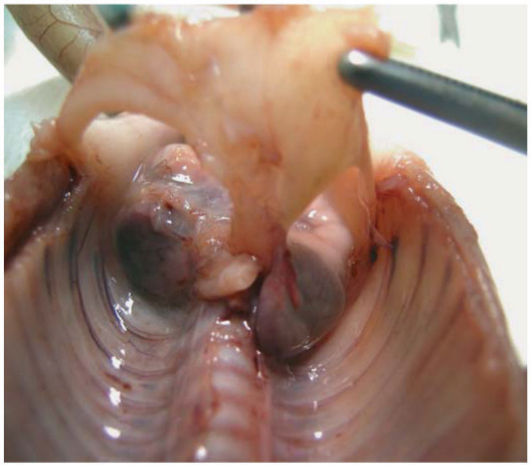


Fig. 3. Necropsic examination (similar features in both fetuses): view from the head of bilateral diaphragmatic defect, more pronounced on the left side.

calized problem but instead as a multiorganic disease, causing a dismal prognosis with survival rates less than 30% [10].

Analysis of a large cohort of consecutively collected cases of CDH showed a relapse rate of 0.7% among siblings [5]. Although recurrence is rare, affected families should be advised that this anomaly is not always sporadic, particularly in cases where associated congenital malformations are found.

Therefore, a thorough investigation is necessary in order to exclude further anomalies. In the case of a twin pregnancy in which one fetus is affected by one structural anomaly, a detailed examination of the other is mandatory because the defects can be subtle and escape the ultrasound diagnosis.

In summary, the etiology, genetic predisposition and subsequent familial inheritance of CDH are not easy to determine, making parental counseling very challenging in multiple pregnancies.

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Capítulo 4

Contributo da autópsia nos casos de interrupção médica da gravidez por anomalias cromossómicas

4.1 Systematic fetal autopsy in cases of medical termination of pregnancy due to chromosomal abnormalities: improving the quality of perinatal care?

Ramalho C, Brandão O, Matias A, Montenegro N. Systematic fetal autopsy in cases of medical termination of pregnancy due to chromosomal abnormalities: improving the quality of perinatal care? Prenat Diag 2010; 30: 1000-1004

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Systematic fetal autopsy in cases of medical termination of pregnancy due to chromosomal abnormalities: improving the quality of perinatal care?

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Objectives Evaluation of the clinical utility of systematically performing autopsy after termination of pregnancy (TOP) due to chromosomal abnormalities.

Methods A retrospective study of cases of TOP due to chromosomal abnormalities was performed between 1 January 2002 and 30 September 2007, in a tertiary referral hospital. A comparative study between prenatal ultrasonographic findings and postmortem findings was performed.

Results There were 57 cases of TOP due to chromosomal abnormalities. The median gestational age at TOP was 17 (range 14–24 weeks). There were 46 trisomies, 4 monosomies, 4 triploidies and 3 cases of structural anomalies. In 25 cases (43.9%) there was at least one major anomaly detected by ultrasound. In 15 cases (26.3%) additional information was obtained after fetal autopsy: in 7 cases further major structural anomalies were detected, and in 8 cases the presence of a major abnormality was not confirmed by the autopsy.

Conclusion Fetal autopsy may provide additional information to the ultrasonographic morphological evaluation of the fetus submitted to TOP due to chromosomal abnormalities. The combination of prenatal ultrasound and pathological examination can broaden our knowledge of the natural history of chromosomal anomalies in the earlier stages of development and eventually contribute to improve the quality of perinatal care. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: chromosomal abnormalities; prenatal ultrasound; prenatal diagnosis; termination of pregnancy; fetal autopsy

INTRODUCTION

During the last decade, chromosomal abnormalities have been increasingly diagnosed prenatally, using fetal cells obtained by invasive techniques (amniocentesis, chorionic villus sampling or cordocentesis). Considering the inherent miscarriage rate of about 0.5 to 2% associated with invasive procedures (Tabor *et al*., 1986; Alfirevic *et al*., 2003; Mujezinovic and Alfirevic, 2007; Tabor *et al*., 2009), these should only be recommended for pregnancies at high risk for chromosomal abnormalities.

Several screening methods, combining ultrasonographic and biochemical markers, have been proposed reaching detection rates of more than 90% of affected fetuses (Wald *et al*., 2003; Malone *et al*., 2005; Nicolaides *et al*., 2005). The majority of fetuses affected by chromosomal abnormalities demonstrate major structural anomalies that can be visualized by prenatal ultrasound examination, which determines the need for an invasive procedure.

Following the diagnosis of chromosomal abnormalities and after genetic counselling, the majority of the couples decide to terminate the pregnancy (Shaffer *et al*., 2006; Quadrelli *et al*., 2007).

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The importance of fetal autopsy is established in cases of medical termination of pregnancy (TOP) due to morphological anomalies (Kaiser *et al*., 2000; Boyd *et al*., 2004; Johns *et al*., 2004; Ramalho *et al*., 2006), but its role and justification in (re)defining chromosomal abnormalities is still under discussion. Since the diagnosis in these cases is definitively established by cytogenetic studies, autopsy is unlikely to change the underlying diagnosis or recurrence risk but may contribute to our understanding of the phenotype. The postmortem examination may also act as an important complementary technique to confirm the presence of anomalies previously detected by ultrasound or reveal additional findings.

The aim of this study was to evaluate the impact of systematically performing autopsies following TOP due to chromosomal abnormalities.

MATERIALS AND METHODS

A retrospective study of TOPs due to chromosomal abnormalities was performed between 1 January 2002 and 30 September 2007, in a tertiary referral hospital. We included cases only until 24 weeks.

Women were referred to our Prenatal Diagnosis Center for advanced maternal age, previous pregnancy affected by a chromosomopathy, major structural anomalies, presence of ultrasonographic markers or

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positive prenatal screening. Some cases were referred for an opinion after the diagnosis of a chromosomal abnormality had been made elsewhere.

Ultrasound scans were performed between 11 and 13 weeks or at the time of referral.

Amniocentesis was performed in all cases. Multiplex ligation-dependent probe amplification (MLPA) for chromosomes 21, 13, 18, X and Y were used, followed by cytogenetic evaluation after amniotic cell culture for all chromosomes.

Following genetic counselling, the couples decided either to continue or to terminate the pregnancy. Termination was performed according to a pharmacological protocol with the additional objective of optimising the preservation of the fetus. According to our department policy, we used vaginal misoprostol. After 22 weeks of gestation, feticide was systematically performed with intravenous fetal analgesia followed by intracardiac KCl.

Parental consent was obtained for autopsy. All cases of TOP were submitted to postmortem examination, at the Developmental Pathology Unit of the Department of Pathology. A team comprising a specialized pathologist and two technicians with special training and interest in perinatal pathology performed all fetal autopsies and placental examination, according to the standard protocols.

Morphological anomalies were classified as major or minor with major anomalies defined as permanent defects that would affect the offspring in a relevant way, both physically and mentally, such as central nervous system anomalies, cardiac defects, gastrointestinal anomalies, renal anomalies, limb defects, facial anomalies, cystic hygroma, hydrops and severe fetal growth restriction.

We compared prenatal with postmortem findings with emphasis on major anomalies. Cases were classified into two groups: those with and without additional morphological information available following autopsy.

We rated chromosomal autosomal abnormalities as 'rare' all except trisomies 13, 18, 21 and polyploidies (Baena *et al.*, 2003).

RESULTS

During this period, 57 elective TOPs were performed due to chromosomal abnormalities until 24 weeks. The median maternal age of the women was 34 years (range 26–44 years). The median gestational age at the time of TOP was 17 weeks (range 14–24 weeks).

The indications for amniocentesis included increased nuchal translucency (20), positive maternal biochemical screening (13), positive integrated screening (8), advanced maternal age (2) and abnormal ultrasonographic findings (10). Four cases were referred to our center after amniocentesis had been performed and the indication for the invasive test was not clear.

In the above-mentioned group, there were 46 trisomies, 4 triploidies, 3 structural abnormalities and 4 sexual chromosomal abnormalities.

Table 1—Chromosomal abnormalities and major anomalies detected by ultrasound ($n = 57$)

—	Major anomalies in ultrasound	
—	Present	Absent
Trisomies	—	—
Trisomy 21	8	26
Trisomy 18	4	2
Trisomy 13	3	0
Trisomy 9	2	0
Trisomy 12	1	0
Monosomies	—	—
Monosomy X	4	0
Triploidies	—	—
69, XXX	2	1
69, XXY	1	0
Structural abnormalities	—	—
46, XX, del (18) (p11.2)	0	1
46, XX, der (5) t (5,7) (15.1; 13) mat	0	1
46, XX, del (17) (p11.2 p11.2)	0	1
—	25	32

In 25 (43.9%) cases there was at least one major anomaly detected by ultrasound (Table 1). The ultrasound scan revealed major anomalies in all cases of trisomy 13, 9 and 12, as well as in 4 cases of monosomy X, 4 cases of trisomy 18 (4/6), 8 cases of trisomy 21 (8/34) and 3 cases of triploidy (3/4).

All fetuses were delivered intact since no surgical approach was needed. The parental consent rate for perinatal autopsy was 100%.

Necropsy detected major anomalies in 23 (40.4%) fetuses. Considering the cases with major anomalies, the difference between the ultrasound and the autopsy findings are not significant ($p = 0.425$).

Necropsy examination provided additional morphologic information in 15 (26.3%) out of 57 cases. Major structural anomalies that were not diagnosed prenatally were identified at autopsy in seven cases (12.2%) (Table 2). Cardiovascular defects, isolated or in association with other major anomalies, were found in five of these cases. In two cases of trisomy 18, there was confirmation of all the defects detected prenatally with additional information. In the two other cases of trisomy 18, in which there were no significant defects detected prenatally, necropsic examination identified major anomalies.

In eight cases (14.0%), the presence of one major anomaly was not confirmed by the autopsy. In four cases of trisomy 21 and in one case of trisomy 13, the presence of suspected cardiac defects was not confirmed. In two cases of trisomy 21 and one case of monosomy X, cystic hygroma was not present at the time of the postmortem examination (which may have represented natural resolution).

Rare chromosomal autosomal abnormalities were diagnosed in six cases (10.5%). These presented a positive screening test at the time of prenatal examination relying on increased nuchal translucency (3), positive maternal biochemical screening (2) and abnormal sonographic findings (1). In this subgroup of aneuploidies,

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Table 2—Autopsy findings not identified at ultrasound examination in fetuses submitted to TOP due to chromosomal abnormalities

Chromosomal abnormality	GA[a]	Confirmed ultrasound findings	Additional postmortem findings
Trisomy 21	19	—	Cardiac defect (atrioventricular septal defect)
Trisomy 18	18	—	Cardiac defect (tetralogy of Fallot), diaphragmatic hernia
Trisomy 18	14	Omphalocele	Limb defect (radial aplasia)
Trisomy 18	16	Fetal growth restriction, subcutaneous edema, limb defect	Cardiac defect (atrioventricular septal defect) and central nervous system anomaly (cerebellar hypoplasia)
Trisomy 18	14	—	Facial defect (cleft lip and cleft palate)
Triploidy	24	Fetal growth restriction	Cardiac defect (atrioventricular septal defect)
46, XX, del (18) (p11.2)	20	—	Cardiac complex defect ('single ventricle')

[a] GA, gestational age at TOP.

the scan revealed major anomalies in trisomies 9 and 12 (Table 1). In the cases with structural chromosomal abnormalities, no major anomalies were detected by ultrasound. In one case with deletion of the short arm of chromosome 18, the autopsy examination identified an unsuspected complex cardiac defect.

DISCUSSION

Although perinatal autopsy rates are decreasing worldwide (Adappa *et al*., 2007), all the cases in our study were submitted to autopsy. In Oxford, from 1991 to 1995 and from 1996 to 2000, the autopsy rate after medical termination fell from 84% to 67% (Kaiser *et al*., 2000), and in Wales, in 1993, the autopsy rate after medical termination was 79% (Cartlidge *et al*., 1995). Interestingly, in this series the frequency of 'rare' autosomal abnormalities is similar to the one reported by Nicolaides *et al*. (2002) (10.4%), and is higher than the one reported by Baena *et al*. (2003) (6.6%).

In these cases of TOP performed for chromosomal abnormalities, there is already a definitive diagnosis. Autopsy, therefore, does not alter the primary diagnosis or its implications, but it may identify additional anomalies that were not recognized prenatally.

In this study, there were seven (12.2%) cases in which a major anomaly, not previously detected, was identified by autopsy. In eight (14.0%) cases, the anomaly detected prenatally by ultrasound was not confirmed by autopsy. This could be explained by the early gestational age at the time of termination, and the obvious limitations of early ultrasound scan, knowing that the diagnosis of some anomalies is only possible in the second trimester of pregnancy (Grandjean *et al*., 1999). In the presence of a chromosomal anomaly, most detected following early pregnancy screening tests, the couples decided to terminate the pregnancy before a detailed late-second trimester morphological ultrasound could be performed. In some cases, only the first trimester ultrasound assessment was carried out.

The identification by autopsy of anomalies not previously detected by ultrasound can also be explained by the absence of systematic evaluation of all organ systems after detection of a major anomaly, in spite of all the morphological ultrasound examinations being performed by specialized doctors working exclusively in this field in our department.

It is difficult to directly compare our study with others because the design of most of them was different. In a study by Isaksen *et al*., (2000) referring to 98 cases with fetal karyotype determined after the detection of anomalies, disregarding minor autopsy findings, the detection rate by ultrasonography was 88%. In six cases, major autopsy findings were not previously detected at the ultrasound examination (Isaksen *et al*., 2000). In another study, performed by Papp *et al*., (2007) dealing with 611 structural malformations diagnosed during autopsy performed in 305 fetuses with chromosomal abnormalities, only 232 (38%) anomalies were detected by ultrasound (Papp *et al*., 2007).

In our study, consistent with previous literature, the group of anomalies more frequently representing 'additional information' was congenital heart defects. The prenatal diagnosis of congenital heart defects is often difficult, with detection rates between 13.7 and 43.8% in a low-risk population (Hafner *et al*., 1998; Stoll *et al*., 1998; Grandjean *et al*., 1999; Garne *et al*., 2001; Acherman *et al*., 2007; Nikkilä *et al*., 2007). Prenatal detection is less effective at lower gestational ages. In a randomised controlled trial, comparing ultrasound examination at 12 or 18 gestational weeks for prenatal detection of major cardiac defects, the prenatal detection rate was low for both policies (11 vs 15%) (Westin *et al*., 2006).

In the current study, additional cardiac anomalies were identified at autopsy in five cases and sonographically suspected cardiac anomalies were not confirmed in five cases. These findings are in accordance with Tennstedt *et al*. (1999) who performed a study of 129 fetuses with prenatal diagnosis of cardiac anomaly and identified additional cardiac anomalies in ten cases by autopsy, while in eight cases the cardiac anomalies diagnosed

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by ultrasound scan were not confirmed (Tennstedt *et al*., 1999).

Hence, it has been clearly demonstrated that the prenatal detection of heart defects is more efficiently performed by a specialist in fetal echocardiography (Stumpflen *et al*., 1996). In all cases of suspected cardiac defect, namely, fetuses affected by trisomy 21, a fetal echocardiography is mandatory to provide further prognostic information since the existence or non-existence of a cardiac defect may influence the ultimate decision of the parents.

In three cases, cystic hygroma was not present at the time of the autopsy examination. This can be explained by the complete spontaneous resolution of cystic hygroma, which can happen either in fetuses with normal karyotype or in fetuses with aneuploidy (Ganapathy *et al*., 2004; Howarth *et al*., 2005). In a study involving 99 cases of cystic hygroma diagnosed prenatally, 11 of the 16 liveborns showed spontaneous resolution (Howarth *et al*., 2005). In a study of 42 cases of cystic hygroma detected between 11 and 23 weeks, spontaneous resolution was seen in 3 of the euploid cases (3 in 17) and in 2 of the aneuploid cases (2 in 20) (Ganapathy *et al*., 2004). It may well be that the only available scan was the one performed in the first trimester of pregnancy, with no subsequent scans for reevaluation.

Decisions concerning TOP in cases of affected fetuses with chromosomal abnormalities are complex and may be influenced by several factors. The prognosis of a chromosomal anomaly is one of the most important determinants of the parental decision (Shaffer *et al*., 2006; Quadrelli *et al*., 2007), and associated ultrasonographic findings play a major role in the couples' choice to terminate or carry on with the pregnancy (Drugan *et al*., 1990; Christian *et al*., 2000; Hamamy *et al*., 2004; Mezei *et al*., 2004). For example, in monosomy X, if cystic hygroma has resolved, and there are not associated anomalies, some parents may choose to continue the pregnancy (Christian *et al*., 2000).

Systematic and critical evaluation of all cases of elective TOP by autopsy is the gold standard for quality control and assessment of the accuracy of prenatal diagnosis. Pathological examination provides additional information to the morphological characterization of the specific chromosomal abnormality in each affected fetus submitted to TOP. The combination of prenatal ultrasound and pathological examination can also improve our knowledge of the evolving phenotype and natural history of chromosomopathies, from early phases of development.

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4.2 Positive biochemical screening for trisomy 18: on the path of trisomy 9

Póvoa A, Ramalho C, Torgal A, Brandão O, Matias A, Oliveira MJV, Montenegro N, Castedo S. Positive biochemical screening for trisomy 18: on the path of trisomy 9. Prenat Diagn 2008; 28: 162–164

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RESEARCH LETTER

Positive biochemical screening for trisomy 18: on the path of trisomy 9

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KEY WORDS: trisomy 9; biochemical screening; prenatal diagnosis

During the last decade, the prenatal diagnosis of chromosomal abnormalities has been improved by the combination of biochemical and ultrasonographic markers. Consistent abnormalities in serum markers have been recognized in trisomy 21, 18, 13, triploidy, and sex aneuploidies. Namely, trisomy 18 is associated with low levels of second trimester α-fetoprotein (AFP) and unconjugated estriol (uE3), low levels of first and second trimester free beta-human chorionic gonadotrophin (β-hCG) and low levels of first trimester pregnancy-associated plasma protein A (PAPP-A). Although some reports refer to abnormal levels of serum markers in trisomy 9 (Lam *et al*., 1998; Roshanfekr *et al*., 1998; Chen *et al*., 2004; Khoury-Collado *et al*., 2004; Quigg *et al*., 2005; Lim *et al*., 2006), a recognizable biochemical pattern for this aneuploidy has not been described yet.

We present two cases of trisomy 9 in which maternal serum screening was positive for trisomy 18.

The first case refers to a 31-year-old healthy primigravida, from a nonconsanguineous couple. There was no family history of congenital malformations. The woman was not under any medication and no recent infections were reported. The first trimester ultrasound, at 11 weeks, showed no anomalies and revealed a nuchal translucency of 1.2 mm. An Integrated test was performed (first sample collected at 12^{+1} weeks for PAPP-A assay and second sample at 15^{+2} weeks for determination of free β-hCG, uE3, AFP, and inhibin A). The screening result was negative for trisomy 21 (at term risk 1/24 000), but positive for trisomy 18 (at term risk >9/10). The MoMs were as follows: PAPP-A 0.16 MoM; free β-hCG 0.19 MoM; uE3 0.44 MoM; AFP 0.82 MoM; inhibin A 1.26 MoM.

Amniocentesis was performed at 16 weeks and the karyotype revealed trisomy 9 in all 15 cells analyzed (47,XY,+9).

The ultrasound performed at 18 weeks of gestation showed oligohydramnios, agenesis of the cerebellar vermis, and dysmorphic features of the hands.

After genetic counseling the parents decided to terminate the pregnancy.

Postmortem examination revealed craniofacial (wide sutures, hypotelorism, small and flattened nose with a small tip, microretrognathia, low-set and malformed ears, high arched palate, bifid tongue), limb (clinodactily with camptodactyly of third and fourth hand fingers, calcaneus-valgus feet), central nervous system (malformed cerebellar vermis), cardiovascular (pulmonary artery stenosis, left iliac primitive artery hypoplasia, and single umbilical artery), genitourinary (renal hypoplasia with both kidneys fused in the midline, small bladder), and other (malrotation of the gut) malformations.

Interphase FISH (fluorescence *in situ* hybridization) analysis of fetal tissues (lung and muscle) and placenta, using a probe specific for the centromere of chromosome 9 (CEP 9 probe Vysis, Downers Grove, IL) and a control probe for the centromere of chromosome 7 (CEP 7 probe Vysis, Downers Grove, IL), revealed trisomy 9 in over 90% of nuclei from all tissues, a percentage not significantly different from the one obtained with the control probe (95%), in keeping with the diagnosis of complete trisomy (47,XY,+9).

The second case refers to a 33-year-old woman, G1, P0, from a healthy and nonconsanguineous couple. There was no family history of congenital malformations. The woman was not under any medication and no recent infections were reported. The first trimester ultrasound, at 11^{+6} weeks, showed no anomalies. At that time a first trimester serum screening test was performed. The screening result was negative for trisomy 21 (at term risk 1/2700), but positive for trisomy 18 (at term risk 1/9), with low β-hCG and PAPP-A levels (0.15 and 0.23 MoM, respectively).

Amniocentesis was performed at 14 weeks and the karyotype revealed trisomy 9 in all 15 cells analyzed (47,XX,+9).

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The ultrasound performed at 15 weeks showed oligohydramnios, fetal bradycardia, clenched hands, and anterior wall defect.

After genetic counseling, the parents opted for termination of pregnancy.

Postmortem examination revealed craniofacial (microcephaly, micrognathia, low-set and malformed ears), central nervous system (semilobar holoprosencephaly), cardiovascular (severe stenosis of pulmonary artery outflow tract, pulmonary arteries hypoplasia, ventricular septal defect), genitourinary (renal hypoplasia), and other (pancreas and thymus hypoplasia, anterior wall defect with gut and liver protrusion) malformations.

Interphase FISH (fluorescence *in situ* hybridization) analysis of fetal tissues (lung and muscle) and placenta, using a probe specific for the centromere of chromosome 9 (CEP 9 probe Vysis, Downers Grove, IL) and a control probe for the centromere of chromosome 7 (CEP 7 probe Vysis, Downers Grove, IL), revealed trisomy 9 in over 89% of nuclei from all tissues, a percentage not significantly different from the one obtained with the control probe (94.5%), in keeping with the diagnosis of complete trisomy (47,XX,+9).

Trisomy 9 is a rare chromosomal disorder that may be present in one of three ways: nonmosaic complete trisomy 9 (an entire extra chromosome 9 is present in all cells), mosaic trisomy 9 (presence of trisomic and disomic cells in a variable proportion), and partial trisomy 9 (trisomy for only a part of chromosome 9). Complete trisomy 9 has a lethal prognosis. In the vast majority of cases, it results in spontaneous abortion in the first trimester. In the rare cases surviving to term, death usually occurs in the early postnatal period. In a review of 15 cases, McDuffie showed that only 3 out of the 12 live borns (25%) lived beyond one week (McDuffie, 1994). Almost 85% of cases occurred in mothers younger than 35 years (McDuffie, 1994). To our knowledge, there is no reported long-term survival for cases with complete trisomy 9.

The characteristic phenotype of complete trisomy 9 includes growth retardation, developmental delay, and craniofacial, cardiovascular, central nervous system,

Table 1—Data on prenatal multimarker serum screening

Cases	Biochemical markers in the second trimester (MoM)	Screening results	Karyotype
Roshanfekr *et al*., 1998	Triple test: hCG 0.2 AFP 0.6 uE3 0.6	+ (trisomy 18)	Trisomy 9
Lam *et al*., 1998	Double test: hCG 0.1 AFP 0.8	+ (trisomy 18)	47,XY,+9 (AF, placenta)
Khoury-Collado *et al*., 2004	Triple test: free β-hCG 0.19 AFP 1.13 uE3 0.26	>1 : 10 (trisomy 18)	47,XY,+9 (AF, placenta, skin, peripheral blood lymphocytes)
Chen *et al*., 2004	Double test: free β-hCG 0.42 AFP 9.2	+ (NTD 1 : 14)	47,XX,+9 (AF, skin)
Quigg *et al*., 2005	Integrated test: PAPP-A 0.54[a] hCG 0.13 AFP 1.77 uE3 0.86 inhibin A 1.01	1 : 30 (trisomy 18)	47,XY,+9 (AF)
Lim *et al*., 2006	Triple test: free β-hCG 0.19 AFP 3.09 uE3 0.39	1 : 5 (trisomy 18) 1 : 18 (NTD)	Trisomy 9
Present study, case 1	Integrated test: PAPP-A 0.16[a] free β-hCG 0.19 AFP 0.82 uE3 0.26 inhibin A 1.26	>9 : 10 (trisomy 18)	47,XY,+9 (AF, placenta, skin)
Present study, case 2	First trimester test: PAPP-A 0.23[a] free β-hCG 0.15[a]	1 : 9 (trisomy 18)	47,XX,+9 (AF, placenta, skin)

[a] In the first trimester.
AF, amniotic fluid; NTD, neural tube defect.

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skeletal, and genitourinary malformations (Yeo *et al*., 2003; Kor-anantakul *et al*., 2006). Kor-anantakul and coworkers reviewed the prenatal sonographic and necropsic findings in 22 cases of trisomy 9 (Kor-anantakul *et al*., 2006). The commonest feature was intrauterine growth restriction that was detected in 85% of second trimester cases. However, this particular feature was not detected in our cases. In both our cases we recorded abnormalities previously described in the literature, including oligohydramnios, agenesis of the cerebellar vermis, and dysmorphic features of the hands. Some of the postnatal findings were not detected prenatally by ultrasound, which was probably due to the scarce amniotic fluid and unfavorable fetal position.

Necropsic examination confirmed some of the sonographic findings and revealed additional phenotypic anomalies in accordance with data previously described in the literature. FISH analysis of fetal tissues and placenta was consistent with the diagnosis of complete trisomy 9, therefore making fetoplacental or fetoamniotic discrepancies unlikely.

The majority of reported cases of trisomy 9 diagnosed prenatally was prompted by the ultrasonic detection of fetal abnormalities. Some reports have mentioned the similarity between the sonographic characteristics of trisomies 9 and 18 (Benacerraf *et al*., 1992; Yeo *et al*., 2003). Information on maternal serum screening values in pregnancies with complete trisomy 9 is scarce. As far as we know, there are only six previously reported cases of trisomy 9 associated with abnormal multimarker screening result (Table 1). All six cases presented with very low levels of hCG or free β-hCG in the second trimester. In one of these cases, high levels of AFP were found in association with an open neural tube defect (Chen *et al*., 2004), a previously reported malformation in complete trisomy 9 (Benacerraf *et al*., 1992). Information concerning first trimester markers was available in only one case (Quigg *et al*., 2005), where low levels of PAPP-A (0.54 MoM) were noted. Four cases were screen positive for trisomy 18, three with the triple test (Roshanfekr *et al*., 1998; Khoury-Collado *et al*., 2004; Lim *et al*., 2006), and one with the Integrated test (Quigg *et al*., 2005).

Our cases screened positive for trisomy 18, one with the Integrated test and one with first trimester serum test. They presented very low levels of free β-hCG (0.19 and 0.15 MoM, respectively) and very low levels of PAPP-A (0.16 and 0.23 MoM, respectively). To the best of our knowledge, we report the first case of a first trimester screening test positive for trisomy 18, which turned out to reveal a fetus with trisomy 9.

More cases are needed to establish whether the combination of low levels of PAPP-A, free β-hCG, and possibly uE3 represent a true serum pattern for trisomy 9.

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4.3 Phenotypic expression in the first case of complete trisomy 12: combination of prenatal ultrasound and necropsic examination

Machado AP, Ramalho C, Loureiro T, Cunha M, Dória S, Carvalho F, Oliveira JP, Brandão O, Matias A. Phenotypic expression in the first case of complete trisomy 12: combination of prenatal ultrasound and necropsic examination. Fetal Diagn Ther 2009; 25: 234–238

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Phenotypic Expression in the First Case of Complete Trisomy 12: Combination of Prenatal Ultrasound and Necropsic Examination

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Key Words
Complete trisomy 12 · Complete trisomy 12, sonographic features · Necropsic examination

Abstract
The authors report the first case of a complete trisomy 12 detected antenatally at 16 weeks of gestation. The ultrasonographic features (craniofacial abnormalities) correlated well with postmortem findings. The absence of the hypophysis gland, olfactory pathways and both adrenal glands, as well as developmental anomalies of the central nervous system, were additional findings at autopsy. The recognition of complete trisomy 12 phenotype can be helpful in the pre- and postnatal diagnosis of future similar cases and parental counseling.
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Introduction

Human trisomy 12 is a rare diagnosis in clinical practice; however, some clinical entities can show this chromosomal abnormality, mostly in a mosaic or incomplete form. Its phenotypic expression depends on the relative amount of affected embryonic cells. Some cases of mosaicism of trisomy 12 were detected pre- and postnatally. Those diagnosed postnatally have an extremely variable clinical presentation and various degrees of severity. A mosaicism of trisomy 12 diagnosed prenatally by chorionic villus sampling may eventually not be confirmed in fetal tissues, suggesting mosaicism confined to the placenta as the most likely explanation [1]. The observation of such an aneuploidy in amniotic cells is an extremely rare event.

The phenotypic features of chromosomal mosaicism are determined by several factors: the particular chromosome involved and its genetic content, the origin of the non-disjunctional error (meiotic or mitotic), the proportion and distribution of abnormal cells, and a potential influence of parental origin and/or of uniparental disomy of the diploid cell line [2]. The cases of mosaicism of trisomy 12 diagnosed in amniocytes show extreme variability: it may range from fetal demise, multiple congenital anomalies, namely congenital heart disease, digit anomalies and craniofacial dysmorphism, to grossly normal liveborns [3]. As expected, a trend towards a direct association between a higher percentage of trisomic 12 cells and the chance of an abnormal outcome was found [3].

Another entity that clinically may share some features with trisomy 12 is the duplication of the short arm of chromosome 12. This chromosomal aberration was de-

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Fig. 1. Ultrasound image of mild enlargement on lateral ventricles.

scribed for the first time in 1973 by Uchida and Lin [4], and until recently, more than 25 cases have been described. There is no clear genotype/phenotype correlation in this syndrome and most cases result from malsegregation of a balanced parental translocation [5]. The 12p trisomy syndrome is very rare, but prenatal diagnosis has been successfully achieved in some cases. It may cause dysmorphic features, dental problems, mental retardation with aggressive behavior and other multiple congenital anomalies [6].

Trisomy 12 is sometimes found in association with chronic lymphocytic leukemia, appearing in 15–30% of cases, and correlating with atypical morphology and shortened survival [7].

Some other tumors are related to trisomy 12, namely Wilms' tumors (WT), one of the commonest kidney tumors in childhood. This tumor presents as a heterogeneous disease with several genetic abnormalities including mutations/deletions of the WT1 gene. Hyperdiploid tumors, mostly related with trisomy 12, may be a unique subgroup of tumors with no WT1 abnormalities [8]. Chromosome 12 has two growth-promoting genes, CCND2 and CDK4, overexpressed in WT, and probably responsible for accelerated proliferation [9].

We present the clinical report of a complete trisomy 12, detailing the phenotypic features associated with this particular form of trisomy, diagnosed prenatally in amniocytes and confirmed later in fetal skin and placental tissues.

Clinical Report

A 30-year-old pregnant woman, gravida 2, para 1, was referred to our center at 16 weeks' gestation after identification of multiple congenital anomalies in a routine ultrasound scan. An increased maternal serum α-fetoprotein (5.05 multiples of the median – MoM) was detected. Levels of free β-human chorionic gonadotropin (0.95 MoM), non-conjugated estriol (0.10 MoM) and inhibin-A (0.83 MoM) were within a normal range for 16 weeks. Her husband was 33 years old, the couple was healthy and non-consanguineous and the family history was unremarkable. The first pregnancy was uneventful and a healthy child was born.

An ultrasound scan was repeated in our unit and it showed a female fetus, with long bones length and abdomen circumference growth according to gestational age and a head circumference above the 95th centile, but with normal shape. Additionally the fetus revealed prominent frontal bones, mild enlargement of lateral ventricles (fig. 1), cerebellar transverse diameter of 15 mm (5th centile) and the nuchal skin fold measured 8.1 mm. A frontal subcutaneous edema, a flattened fetal face profile and slightly shortened nasal bones (3.8 mm) were also observed, suggesting a midfacial hypoplasia (fig. 2). The heart showed two echogenic foci in the left ventricle and one in the right ventricle. Amniotic fluid volume was within the normal range and the placenta was 2.1 mm thick with normal morphology.

Amniocentesis was performed to clarify these findings and the amniocyte culture revealed a fetal karyotype of 47,XX,+12 in all 30 metaphases studied. The couple asked for elective termination of pregnancy at 19 weeks' gestation. Autopsy revealed a female fetus with growth parameters according to gestational age and multiple congenital anomalies (table 1). The pathological study of the placenta showed no abnormalities. Fetal skin and placenta fragments were collected after expulsion and the presence of a karyotype 47,XX,+12 was demonstrated in both tissues.

Discussion

Although the variable phenotypic expression of the different forms of trisomy 12 is well known, the particular characteristics of the complete form are not available in the literature. It is very difficult to predict the phenotypic characteristics of such an aneuploidy. The great amount of genetic material that exists in chromosome 12 can lead to multiple different anomalies. Alternatively, the complete trisomy can lead to a blocking process of genetic material that can be expressed with the characteristics of a monosomy.

The multiple congenital anomalies found are probably related to the chromosomal abnormality, some of them already expressed in other similar syndromes, namely trisomy 12 mosaicism and trisomy of the short arm of chromosome 12 (table 2). Although congenital heart anomalies are one of the most frequent internal malfor-

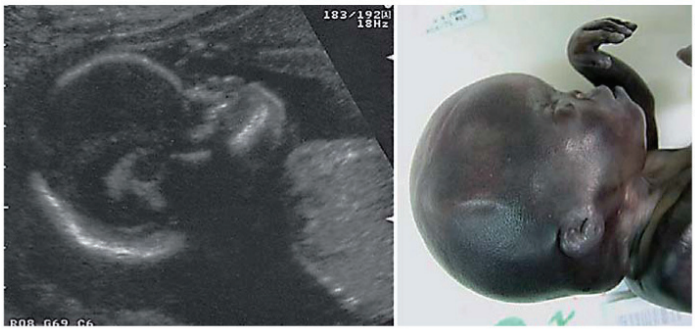


Fig. 2. Ultrasound and necropsic images of midfacial hypoplasia.

Table 1. Sonographic and necropsic findings in the reported case

	Sonographic findings	Necropsic findings
External features		
Body	Nuchal skin fold	Nuchal skin fold
	Short neck	Short neck
		Moderate thoracolumbar kyphosis
		11 pairs of ribs
Head and face	Prominent frontal bones	Prominent frontal bones
	Nasal bone hypoplasia	Nasal bone hypoplasia
	Prognathism	Prognathism
		Low-set ears
		Severe hypoplastic nares
		Hypertelorism
Extremities		Bilateral clinodactyly (2nd, 3rd, 4th and 5th fingers)
		Preaxial bilateral hexadactyly (hallux duplication)
		Partial syndactyly of the duplicated hallux
Internal features		
Central nervous system	Dilatation of lateral ventricles	Absence of olfactory pathways
	Hypoplastic cerebellum	Absence of hypophysis gland
		Hypoplastic corpus callosum
		Hippocampus caudally dislocated, with abnormal morphology
		Thalamic area dislocated and malformed
		Moderate dilatation of lateral ventricles and 3rd ventricle – hydrocephalus
		Hypoplastic cerebellum
		Mild hypoplastic brainstem
		Abnormal olivary nuclei
Others	Heart echogenic foci	Larynx hypoplasia
		Thyroid hypoplasia
		Lung hypoplasia and relative immaturity (both lungs with 2 lobes)
		Kidney hypoplasia
		Complete absence of adrenal glands

Table 2. Clinical features described for the present case and comparison with mosaicism of trisomy 12, partial trisomy 12p, Pallister-Killian and acrocallosal syndromes

	Mosaicism of trisomy 12	Partial trisomy 12p	Pallister-Killian	Acrocallosal	Present case
Prominent forehead	+	+	+	+	+
Hypertelorism	+	+	+	+	+
Low-set ears	–	+	+	–	+
Short nose	+	+	+	–	+
Flat nasal bridge	+	+	+	–	+
Hypoplastic nares	–	+	+	–	+
Long philtrum	+	+	+	–	–
Macroglossia	–	–	+	–	–
Everted lower lip	–	+	–	–	–
Short neck	+	+	+	–	+
Hallux duplication	–	+	+	+	+
Absence of corpus callosum	–	+	+	+	+
Congenital heart disease	+	+	–	–	–



Color version available online

Fig. 3. Necropsic image of adrenal glands absence.



Color version available online

Fig. 4. Necropsic and radiologic images of hallux duplication.

mations associated with the former entities [2, 3, 10, 11], the fetus described in the present report did not present this particular abnormality.

The most relevant anomalies were found in the central nervous system and the face. These craniofacial abnormalities are described for both monosomy 12p and trisomy 12p [11]. All craniofacial anomalies found in the present fetus were already described in this context. The prognathism, hypertelorism, short nose, prominent forehead and short neck are features also associated with Pallister-Killian syndrome, a dysmorphic condition caused by mosaicism for tetrasomy 12p [12].

Another relevant finding was the absence of the hypophysis. This anomaly can be the cause of several endocrine abnormalities found in our clinical report. One may wonder if it could be implicated in thyroid hypoplasia and adrenal glands absence (fig. 3) [13]. The association of hallux duplication (fig. 4), hypertelorism and corpus callosum hypoplasia was already described in both trisomy 12p and tetrasomy 12p, and is named acrocallosal syndrome [14]. This syndrome, first described in 1979 by Schinzel and Kaufmann [15], is a rare congenital disorder with no specific genetic cause identified so far. It occurs sporadically or, in some families, with an autosomal recessive pattern of inheritance. The similarity of this entity with trisomy 12p and tetrasomy 12p remains unclear and we may wonder if there are one or more genes in chromosome 12 that, if duplicated or disrupted, cause ac-

rocallosal syndrome. At present the disorder can only be identified by clinical symptoms.

Chromosome 12 presents some loci that play an important role in the neuronal differentiation. The *LHX5* gene, localized to chromosome 12q24.31–24.32, is essential for the neuronal differentiation and migration during development of the hippocampus formation. An excess of that gene product can interfere with the normal migration process and lead to the abnormal position and morphology of the thalamus, hypothalamus and base nuclei, and interfere with neuronal cortication, with olfactory pathways and with the hippocampus itself [16].

Trisomy of chromosome 12 seems to affect the early embryonic development, resulting in congenital anomalies and dysmorphic features, but genes implicated in brain development, important in later stages of pregnancy, are also disturbed. The amount of affected cells may be implicated in neurological and dysmorphic outcome [6], which could explain the variety of anomalies observed in mosaicism, partial trisomy or complete trisomy.

The high levels of α-fetoprotein detected in the maternal serum may result from one of the multiple anomalies or from the abnormal karyotype [17] and were already described in association with mosaicism of trisomy 12 [3]. Some of the anomalies detected at autopsy were not seen in the ultrasound scan probably because early gestational age limited the visual accuracy and most of the abnormalities were very subtle and of difficult diagnosis. We reinforce the importance of the necropsic examination performed by a fetal pathologist to assess the quality of the prenatal diagnosis [18].

In the future we hope to have the possibility of comparing this clinical report with others describing a similar entity, in order to understand what is clinically relevant to the diagnosis of this disease. The combination of sonographic and necropsic findings provides useful information to improve the knowledge of this syndrome and to refine parental counseling.

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Capítulo 5

Variabilidade fenotípica na síndrome de Down

5.1 Phenotypic variability in fetuses with Down syndrome: a case-control pathological evaluation

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Phenotypic Variability in Fetuses with Down Syndrome: A Case-Control Pathological Evaluation

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Key Words
Down syndrome · Fetal pathology · Morphological characteristics · Necropsy

Abstract

Objectives: Evaluation of morphological and morphometric characteristics of fetuses with Down syndrome based on a detailed postmortem examination and comparison of these fetuses with a control group of fetuses with normal karyotype to improve the prenatal knowledge of Down syndrome characteristics. ***Material and Methods:*** A case-control study was undertaken in a tertiary referral hospital. The Down syndrome fetuses (50) were compared with chromosomally normal fetuses (47) matched for gestational age. Biometric and morphological parameters were compared. ***Results:*** The biometry of fetuses with Down syndrome differs from control fetuses only in the occipitofrontal diameter and in the nasal bone length. There were craniofacial anomalies in 96% of fetuses with Down syndrome and in 51% of fetuses with normal karyotype. With the exception of small ears, all craniofacial features analyzed were significantly different between Down syndrome fetuses and control fetuses. All extremity features of fetuses with Down syndrome were significantly different from fetuses without Down syndrome. There were 31 fetuses with Down syndrome (62%) with 55 internal anomalies. Nineteen fetuses (38.8%) showed 27 cardiac anomalies. Six fetuses had more than one anomaly. There were 18 fetuses (36%) with 28 noncardiac anomalies. ***Discussion:*** The importance of studying the morphological characteristics of fetuses with Down syndrome is the contribution to a better knowledge of fetal characteristics and probably to optimize prenatal diagnosis.

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Introduction

Down syndrome (DS; MIM No. 190685) is the most common chromosomal abnormality reaching viability. It is caused by the triplicate state (trisomy) of all or of a critical portion of chromosome 21. In most of the cases the chromosomal anomaly found in Down syndrome is trisomy 21 (95%). Robertsonian translocations account for approximately 4% of cases and mosaicism for 1%. There is an estimated prevalence of 1.6–1.8 in 1,000 live births [1, 2].

Prenatal diagnosis requires invasive procedures (amniocentesis, chorionic villus sampling or cordocentesis), with a risk of miscarriage of less than 1% [3–5]. Noninvasive prenatal diagnosis is an active research area, but until now this cannot be used routinely in clinical practice. During the last few years, extensive research has identified several biochemical and ultrasonographic markers

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associated with Down syndrome in the first and second trimesters. Different screening methods have been developed. It is now possible to identify fetuses with Down syndrome by the combination of these markers with a detection rate of 93–96% with a false-positive rate of 5% [6, 7].

Down syndrome is associated with a rich phenotypic variability. Most of the phenotypic features are variable in both prevalence and expression. Flat facial profile, flat occiput, upward-slanting palpebral fissures, low-set ears, small ears, micrognathia, protruding tongue, nuchal fold thickness, single transverse palmar crease, and plantar cleft between first and second toes, are characteristics present in 38–88% [8]. Two exceptions are the presence of mental retardation and neonatal hypotonia in most affected individuals. Individuals with Down syndrome are known to have a high frequency of birth defects, particularly affecting the cardiac and gastrointestinal systems. Congenital cardiac abnormalities, namely atrioventricular canal defects and ventricular septal defects are present in 42–56% of Down syndrome [9–11]. Gastrointestinal abnormalities, namely duodenal atresia and anal atresia are present in 11% [9]. However, the majority of the studies reporting abnormalities associated with Down syndrome are based on the late evaluation of children and adults. Until now, few studies have dealt with the morphological characteristics of fetuses with Down syndrome [12]. Some have only evaluated specific anomalies [13, 14] and others are solely based on prenatal ultrasonographic findings [15, 16].

The aim of this study was to evaluate the morphological and morphometric characteristics of fetuses with Down syndrome based on a detailed postmortem examination and to compare these fetuses with a control group of phenotypically normal fetuses.

Material and Methods

A case-control study was undertaken in a tertiary referral hospital. All cases of fetuses with Down syndrome submitted to autopsy between 1/1/2002 and 31/12/2008 were reviewed. The Down syndrome fetuses were compared with chromosomally and morphologically normal fetuses matched for gestational age. The study was approved by the Ethics Committee of S. João Hospital.

The 50 cases evaluated were fetuses either submitted to termination of pregnancy (n = 47) or with spontaneous intrauterine fetal death (n = 3). Prenatal diagnosis (n = 48) was performed by amniocentesis in 47 cases and by chorionic villus sample in one case. The evaluation was performed by multiplex ligation-dependent probe amplification (MLPA) for chromosomes 21, 13, 18, X and Y, and by cytogenetic evaluation after amniotic cell culture for all chromosomes. The postmortem diagnosis was based on karyotype obtained after skin culture (n = 2).

The indication for prenatal diagnosis included increased nuchal translucency (n = 21), positive maternal biochemical screening (n = 8), positive integrated screening (n = 10), advanced maternal age (n = 3), repeated embryo-fetal losses (n = 1) and abnormal ultrasonographic findings (n = 2). Three cases were referred to our center after amniocentesis had been performed and the indication for the invasive test was not clear.

Following genetic counselling, the couples decided to continue or to terminate the pregnancy. The termination was performed with vaginal misoprostol according to department policy [17]. After 22 weeks of gestation, termination was systematically preceded by feticide under fetal analgesia.

The controls (n = 47) were selected using the following criteria for normality: (1) agreement between gestational age and morphological data which was used as the gold standard for fetal age; (2) absence of major malformations and absence of 3 or more minor anomalies (normal variants); (3) well-documented acute placental (abruption or infection) or umbilical cord cause (circular of umbilical cord) for fetal death. All controls had normal karyotype or exclusion of trisomy 21 by MLPA.

Parental consent was always obtained before autopsy. All cases were submitted to postmortem examination at the Developmental Pathology Unit of the Department of Pathology. A team of a pathologist and two technicians with special training and totally dedicated to fetal and perinatal pathology performed all the fetal autopsies and placental examination, according to standardized procedures. The protocol for autopsy has been adapted, modified and improved, over time, according to clinical requirements [18–23]. Besides the systematic approach using standard weights, measurements (namely those specifically related to dysmorphology) recorded at an appropriate protocol, and photographic documentation are performed. Conventional X-ray and Faxitron examinations were undertaken. The malformations, in general, are checked in situ, while the anatomic relationships are preserved. This is extremely important when dealing with congenital heart disease [24]. If there is autolysis, a few hours of fixation are required before proceeding with the dissection, followed by evisceration. The evisceration takes place by removing blocks of organs. Finally, sections are taken, routinely, at the time of the gross necropsic examination for histological study. Two observers (an obstetrician and a pathologist) performed the reevaluation of all cases.

In both groups the following biometrical evaluations were performed: measurements of weight, crown-rump length, foot and hand length, brain weight and transverse cerebellar diameter. The presence or absence of the following external morphological characteristics were evaluated: flat facial profile, flat occiput, upward-slanting palpebral fissures, low-set ears, small ears, micrognathia, protruding tongue, nuchal fold thickness, single transverse palmar crease, and plantar cleft between first and second toes. On the X-ray or on the Faxitron the following biometrical and morphological skeletal evaluations were performed: measurements of the diaphyseal length of femur and humerus, the biparietal (BPD) and occipitofrontal diameters (OFD), and the nasal bone length (NBL). The morphological appearance of the middle phalanx of the fifth digit and its length were compared to those of the terminal phalanx of the same digit (from 16 weeks on). The number of ribs, the presence of clinodactyly of the fifth digit and the presence of camptodactily were also evaluated.

The gestational age was calculated by the crown-rump length measurement in the first trimester ultrasound, and confirmed on

Table 1. Biometry of fetuses with Down syndrome and chromosomally normal fetuses

	With Down syndrome mean (SD)	Without Down syndrome mean (SD)	p value (Student's t test)	95% CI
Body weight, g (n = 48/45)	169.4 (104.2)	168.2 (100.4)	0.955	–41.012 to 43.399
Femur length, mm (n = 48/47)	21.6 (6.0)	23.5 (6.2)	0.132	–0.438 to 0.585
Humerus length, mm (n = 46/47)	21.3 (5.2)	22.9 (5.6)	0.168	–0.378 to 0.666
Crown-rump length, mm (n = 48/46)	137.3 (27.8)	138.8 (26.2)	0.795	–1.252 to 0.962
Biparietal diameter, mm (n = 43/38)	37.7 (7.2)	37.8 (6.8)	0.964	–0.320 to 0.306
Occipitofrontal diameter, mm (n = 26/40)	43.0 (7.1)	48.0 (7.8)	0.011	–0.876 to –0.119
BPD/OFD (n = 24/35)	0.890 (0.224)	0.794 (0.078)	0.022	0.014 to 0.171
Foot length, mm (n = 49/47)	24.5 (6.9)	24.9 (6.3)	0.769	–0.386 to 0.227
Hand length, mm (n = 48/45)	19.7 (4.7)	21.7 (5.2)	0.051	–0.408 to 0.001
Brain weight, g (n = 42/35)	21.2 (5.2–83.0)*	23.5 (7.5–97.5)*	0.570†	
Brain weight/body weight (n = 42/35)	0.131 (0.13–0.23)*	0.150 (0.12–0.31)*	0.002†	
Transverse cerebellar diameter, mm (n = 27/20)	16.6 (1.8)	16.9 (2.6)	0.668	–1.570 to 1.015
Nasal bone length, mm (n = 45/46)	0.0 (0.0–6.7)*	3.0 (0.0–6.0)*	<0.0005†	

SD = Standard deviation; * median (minimum–maximum); † Mann-Whitney U.

autopsy by evaluation of several biometric parameters, as well by estimation of organ maturation. BPD/OFD ratio and brain weight/body weight ratio were calculated. Brachycephaly was defined as BPD/OFD ratio >0.872 [13]. Hypoplasia of nasal bone was defined as NBL <3 mm [25], and absence of nasal bone was considered whenever the nasal bone could not be seen either on X-ray or on Faxitron. We defined short femur or humerus whenever measurements of the diaphyseal length of femur and humerus were inferior to the 5th centile [26, 27]. The presence of internal structural anomalies was evaluated in fetuses with Down syndrome.

Statistical analysis was performed with SPSS Statistics 17.0 (SPSS Inc., Chicago, Ill., USA). Normally distributed continuous variables were reported by mean and standard deviation, and continuous variables with skewed distribution were reported by median and range (minimum and maximum). Student's t test (for independent samples) was used to evaluate the significance of differences between means of normally distributed continuous variables. The Mann-Whitney U test was used to evaluate the significance of differences between groups in continuous variables with skewed distribution. The χ^2 test or Fisher's exact test was used to determine the two-tailed statistical significance of differences between proportions in 2×2 tables (categorical variables). For all tests, a p value of <0.05 was considered significant.

Results

Mean maternal age of the women in the Down syndrome group (35.3 ± 4.4 years), was superior to the mean maternal age of the women in the control group (31.8 ± 7.0 years) (p = 0.004).

In total, 50 fetuses with Down syndrome were evaluated, 49 with full trisomy 21 and one case with a mosaic trisomy 21 (47,XX,+21[16]/46,XX[13]). There was no difference in mean gestational age (17.3 ± 1.9 weeks vs. 17.4 ± 2.0 weeks) (p = 0.676). The proportion of masculine and feminine fetuses in the two groups was not different: 26 (52.0%) male and 24 (48.0%) female fetuses in the Down syndrome group, and 22 (46.8%) male and 25 (53.2%) female fetuses in the control group (p = 0.609).

The biometry of fetuses with Down syndrome differs from control fetuses only in the OFD and in the NBL (table 1). The OFD and NBL were significantly smaller in trisomic fetuses. The BPD/OFD ratio was statistically higher in Down syndrome compared to the control group. The brain weight/body weight ratio was statistically lower in Down syndrome fetuses (table 1).

There were craniofacial anomalies in 96% of fetuses with Down syndrome and in 51% of chromosomally normal fetuses (one or two minor anomalies) ($p < 0.0005$). With the exception of small ears, all craniofacial features analyzed were significantly different between Down syndrome fetuses and control fetuses (table 2).

All extremity features of fetuses with Down syndrome were significantly different from fetuses without Down syndrome (table 2). No significant differences in the finding of less than 24 ribs and single umbilical artery were also observed (table 2).

Table 2. Features of fetuses with Down syndrome and chromosomally normal fetuses

	With Down syndrome, n (%)	Without Down syndrome, n (%)	p value
Craniofacial features			
Brachycephaly (n = 24/35)	14 (58.3)	1 (2.9)	<0.0005†
Flat facial profile (n = 47/43)	27 (57.4)	5 (11.6)	<0.0005†
Flat occiput (n = 47/43)	37 (78.7)	8 (18.6)	<0.0005*
Micrognathia (n = 49/43)	17 (34.7)	4 (9.3)	0.005†
Upward-slanting palpebral fissures (n = 49/43)	6 (12.2)	0	0.028†
Absent/hypoplasic nasal bone (n = 45/46)	36 (80.0)	20 (43.5)	<0.0005*
Protruding tongue (n = 49/43)	8 (16.3)	0	0.006†
Low-set ears (n = 50/42)	26 (52.0)	4 (9.5)	<0.0005*
Small ears (n = 49/43)	5 (10.2)	0	0.058†
Nuchal fold thickness (n = 50/47)	38 (76.0)	7 (16.7)	<0.0005*
Extremity features			
Short femur (n = 48/47)	28 (58.3)	2 (4.3)	<0.0005†
Short humerus (n = 46/47)	14 (30.4)	0	<0.0005†
Broad short hands (n = 44/47)	26 (59.1)	0	<0.0005†
Brachydactyly (n = 44/47)	27 (61.4)	0	<0.0005†
Single transverse palmar crease (n = 50/47)	22 (44.0)	0	<0.0005†
Campodactyly (n = 50/47)	10 (20.0)	2 (4.3)	0.028†
Fifth-finger clinodactyly (n = 49/46)	21 (42.0)	7 (15.2)	0.004*
Absent/hypoplasic fifth finger mid-phalanx (n = 33/32)	25 (75.8)	1 (3.1)	<0.0005†
Drum-like finger (n = 49/47)	17 (34.7)	0	<0.0005†
Sandal gap (n = 50/47)	7 (14.0)	0	0.013†
Plantar cleft (n = 50/47)	10 (20.0)	1 (2.1)	0.008†
Other features			
Less than 24 ribs (n = 46/47)	15 (32.6)	11 (23.4)	0.362*
Single umbilical artery (n = 50/46)	1 (2.0)	1 (2.2)	1.000†

* χ^2 test; † Fisher's exact test.

The internal anomalies of fetuses with Down syndrome detected at necropsic examination are discriminated in table 3. There were 31 fetuses (62%) with 55 internal anomalies. Nineteen fetuses (38.8%) showed 27 cardiac anomalies (table 3). Six fetuses had more than one anomaly. Four fetuses had atrioventricular septal defect (two complete and two incomplete forms) and one fetus had truncus arteriosus communis. Eighteen fetuses (36%) showed 28 noncardiac anomalies (table 3). There were two cases of microcephaly with microencephaly. In six fetuses cardiac and noncardiac anomalies coexisted. One fetus with normal karyotype had an isolated right subclavian artery.

Discussion

The phenotype of Down syndrome can be recognized in a fetus as early as 14 weeks' gestation [28], but Keeling considered that fetuses with trisomy 21 in the second trimester could not yet have developed the typical abnormalities of the face and head; indeed, some fetuses appear normal even to the experienced observer [20].

The OFD and NBL are the only biometric parameters significantly different between fetuses with Down syndrome and fetuses without Down syndrome. The OFD was significantly smaller in trisomic fetus and the BPD was higher but without significant difference. The BPD/OFD ratio was statistically higher than in controls. This agrees with a previous report by Borenstein et al. [30] which states the impairment in growth of the OFD is more marked than that of the BPD, and consequently the BPD/OFD ratio is increased. Using the threshold value of 0.872 proposed by Stempfle et al. [13], brachycephaly was present in 63.6% of the cases. These observations confirm that brachycephaly is an early manifestation in Down syndrome [13, 14, 30].

Body and brain weight were not different between two groups, but the brain weight/body weight ratio was sta-

tistically lower in Down syndrome fetuses. This is in accordance with Guihard-Costa et al. [14], who in a postmortem study, comparing 922 normal with 355 fetuses with trisomy 21, had observed impairment of brain growth in Down syndrome fetuses. In the study by Guihard-Costa et al. [14], the impairment of brain growth was observed as early as the beginning of the second trimester. On the contrary, Schmidt-Sidor et al. [31] did not find any differences in skull shape, brain growth and brain maturation in Down syndrome and non-Down syndrome fetuses, aged 15–22 weeks of gestation. Brain weight is usually in the low normal range, but in our group there were two cases of microcephaly with microencephaly.

In agreement with previous postmortem radiologic studies [13, 32–34], the NBL was significantly smaller in fetuses with Down syndrome. The number of fetuses with absent or hypoplastic nasal bone was significantly higher in the Down syndrome group than in the control group (80.0 vs. 43.5%). The prevalence of absent or hypoplastic nasal bone was superior to the prevalence reported in ultrasound evaluations, in both groups. The results from six studies comparing NBL in trisomy 21 and normal fetuses, showed a combined prevalence of absent or hypoplastic nasal bone of 60% and 1.4% [25]. The higher number of fetuses with absent or hypoplastic nasal bone, in both groups, in our study, could be due to a methodological problem. The voltage and exposure time used in radiological evaluation might have been insufficient for the gestational age of the fetuses. Absent or hypoplastic nasal bone is actually used in the risk assessment for trisomy 21 in the first and second trimesters of pregnancy [25, 35, 36].

The number of fetuses with craniofacial anomalies (96%) was in agreement with previous studies. In the descriptive study by Grangé et al. [12] there were craniofacial anomalies in 87% and Szigeti et al. [15] found facial anomalies in 91% fetuses with trisomy 21 by necropsic study.

Newborns and adults with Down syndrome have short ears [37], and prenatal studies showed a statistically significant difference between the ear sizes of normal fetuses and fetuses with trisomy 21 but only with a small degree of deviation from normal [38–40]. Probably due to this tiny difference, small ears were the only craniofacial feature not significantly different between Down syndrome and control fetuses in our study.

Flat occiput was the second most prevalent alteration in fetuses with Down syndrome, in agreement with the initial descriptions [41]. Nuchal edema is associated with Down syndrome since Benacerraf et al. [42, 43] in 1985 described this alteration in ultrasound in the second trimester. Presently it is one of the most important second trimester markers with an odds ratio of 15 [44]. Comparing our results (76%) with others, they are more expressive than the results from Szigeti et al. [15] (21%). The evaluation of nuchal edema in ultrasound is an objective parameter, whilst in postmortem examination it is more subjective. This could explain the higher number of fetuses with nuchal edema reported in our study.

We did not find significant differences between the length of long bones (femur and humerus) in fetuses with and without Down syndrome, in agreement with the work of Stempfle et al. [13]. We cannot exclude the bias implicated by the small size of our series. When we consider a short femur or humerus, those are significantly more prevalent in fetuses with Down syndrome. It is known since the 1980s that Down syndrome is associ-

Table 3. Anomalies in fetuses affected by Down syndrome

Anomaly	n (%)
Cardiovascular anomalies	
Ventricular septal defect	6 (12)
Atrioventricular septal defect	4 (8)
Truncus arteriosus communis	1 (2)
Dilated right auricle	1 (2)
Dysplastic tricuspid valve	1 (2)
Bicuspid aortic valve	1 (2)
Absent ductus arteriosus	1 (2)
Hypoplastic main pulmonary artery	1 (2)
Myocardial calcifications	8 (16)
Aberrant left subclavian artery	1 (2)
Persistent left superior vena cava	2 (4)
Central nervous system	
Microcephaly with microencephaly	2 (4)
Renal	
Hydronephrosis and hydroureter	2 (4)
Pyelectasia	3 (6)
Microcystic dilatation of renal tubules	2 (4)
Hypoplasia	1 (2)
Gastrointestinal	
Intestinal calcifications	5 (10)
Intestinal malrotation	4 (8)
Meckel's diverticulum	1 (2)
Bilobular spleen	1 (2)
Limb defects	
Equinovarus	4 (8)
Fetal hydrops	1 (2)
Cystic hygroma	1 (2)
Adrenal hypoplasia	1 (2)

ated with short femur and/or humerus and those markers could be used in association with other signs in the prenatal detection of trisomy 21 [45, 46]. The humerus is the most commonly affected bone [47], but in our series the prevalence of short femur is superior to the prevalence of short humerus. When we compared our results with the evaluation from others at autopsy, our prevalence of short femur is superior. FitzSimmons et al. [47] detected short femur in 8% and short humerus in 28% of fetuses with Down syndrome, while Grangé et al. [12] showed a prevalence of short femur of 15%, and Szigeti et al. [15] a prevalence of short femur/humerus of 7%. Those differences can be due to the criteria used to define shortened length.

The fetuses with Down syndrome present broad short hands and brachydactyly, but there are few studies evaluating the prevalence of those anomalies in these fetuses. We found broad short hands in 59.1% and brachydactyly in 61% of fetuses with Down syndrome. Kava et al. [48] found brachydactyly in 11.1% of Down syndrome in a postnatal evaluation.

Absent or hypoplastic fifth finger mid-phalanx is the most prevalent anomaly of extremities in our group. Our group had 75% fetuses with absent or hypoplastic fifth finger mid-phalanx, superior to the results of the radiologic study by Stempfle et al. [13], who found 35.5% fetuses with no ossified middle phalanx of the fifth digit before 23 weeks. Our results are similar to those described by Grangé [12]. The middle phalanx of the fifth finger is normally the last to be ossified, and in Down syndrome fetuses this event is delayed. There is a pathological delayed maturation that affects all the bones of the hand, which is more pronounced in the phalanges [49]. Our results are in accordance with that from Hall [50] who found dysplastic middle phalanx of the fifth finger in 60% of the Down syndrome fetuses.

There was no difference in the prevalence of single umbilical artery between Down syndrome and chromosomally normal fetuses, in contradiction with the results from the population-based study (with 2.5 million infants including 2,894 Down syndrome infants) by Torfs and Christianson [9], who observed that the presence of single umbilical artery was increased 18.6-fold among Down syndrome births. The values observed for single umbilical artery in Down syndrome fetuses in our series was similar to the values from other postmortem fetal studies [12, 15]. Lubusky et al. [51], in a prospective study, found a prevalence of single umbilical artery of 12.8% in the Down syndrome group and 4.1% in the group of chromosomally normal fetuses.

We did not find significant differences in the reduced number of ribs. The number of fetuses with Down syndrome with less than 24 ribs (32.6%) is similar to the results by Edwards et al. [52] (37%). To our surprise we identified a higher number of normal fetuses with this alteration (23.4%) when comparing with other studies (5.2–8%) [52–54]. In a post-mortem radiological study (spontaneous and medical termination), 11 ribs were observed in 10.1% (38.5% of them had chromosomal abnormalities) [55].

In our study, we found 62% of Down syndrome fetuses with internal anomalies. This value is higher than that reported by Fabia and Drolette [56] (35.6%). The most common malformations found in Down syndrome are cardiac defects. In the present study, 38.8% of the fetuses had cardiac anomalies. Our rate of cardiac anomalies is similar to the one reported by Freeman et al. [57] (44%), Grangé et al. [12] (42%) and Stoll et al. [11] (44.6%), but lower than the one reported by Torfs and Christianson [9] (55.9%) and Hyett et al. [58] (53%), and higher than the values reported by Kallen et al. [59] (26%) and by Szigeti et al. [15] (23.4%). Despite the difficulty in comparing the presence and type of cardiac defects, due to their definition, atrioventricular septal defect was the most frequent type of cardiac defect in our series (20%), in agreement with other reports [9, 11, 57–59]. We had one case of truncus arteriosus that is usually not associated with Down syndrome [9].

Gastrointestinal defects are the second most common defects reported as being associated with Down syndrome with a prevalence of 6.7–10.9% [9, 10]. In our study, intestinal malrotation was present in 8% of fetuses, higher than the rates reported by Torfs and Christianson [9] (1.5%) and by Fabia and Drolette [56] (0.12%). We do not have any case of atresia or stenosis of small intestine or duodenal atresia, the most frequently described gastrointestinal defects occurring in Down syndrome (4.8% intestinal, 4.5% duodenal) [9, 59].

We know that it is clinically easy to suspect Down syndrome in infants but the diagnosis may be difficult in the neonatal period [60]. Moreover, in the fetal period characteristics are not clearly identified.

Our data in fetuses affected by Down syndrome clarify the natural history of fetal characteristics. This background knowledge is undoubtedly a major contribution to optimize prenatal diagnosis. With better knowledge of fetal phenotype, valuable information can be added to prenatal markers of Down syndrome in order to decrease false-positive rate and enhance prenatal identification.

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Capítulo 6

O contributo da autópsia perinatal nas anomalias cardíacas congénitas

6.1 Cardiac findings in routine fetal autopsies: more than it meets the eye?

Ramalho C, Brandão O, Monterroso J, Matias A, Montenegro N. Cardiac findings in routine fetal autopsies: more than it meets the eye? Submitted

Abstract

Introduction: The main objective of this study was to evaluate the spectrum of cardiac anomalies found in the routinely performed fetal autopsies and to establish the correlation between prenatal and postmortem diagnosis.

Material and methods: A retrospective study of fetal autopsies was performed in a tertiary referral hospital. All cases with cardiac anomalies were analyzed. The situs was classified as normal or abnormal. Seven main categories were established and each case was assigned to a single group. Cardiac defects were also classified as isolated or with associated anomalies. In the cases with prenatal diagnosis, we performed a correlation between prenatal and postmortem findings.

Results: Abnormal cardiac findings were identified in 99 fetuses (13.6%). Prenatal diagnosis of cardiac anomaly was done in 50 cases (50.5%). There was situs anomaly in four (4.2%). The two most common categories were septal defects and complex anomalies, occurring in 21 fetuses (21.2%). Associated anomalies were found in 67 fetuses (67.7%). Septal anomalies was the only category of anomalies more frequent in cases with associated anomalies than in cases with isolated anomalies (p=0.012). There was complete agreement between prenatal and postmortem diagnosis in 36 cases (72%), and major agreement with additional information in ten (20%). When the echocardiogram was not performed by a specialist, the number of cases classified with complete disagreement was superior (33.3% vs 2.4%) (p=0.002).

Discussion: The high prevalence of cardiac defects in lost pregnancies, some of them lacking prenatal diagnosis, highlights the importance of examining the heart in all cases.

Introduction

Congenital cardiac anomalies are the most common structural defects in newborns, with a birth incidence ranging from 3.3 to 8.1 per 1000[1-5]. Its incidence in stillbirths is higher (27.5 per 1000) than in live births (5.6 per 1000)[5].

The prognosis of congenital cardiac anomalies is poor and they contribute significantly to infant mortality. Cardiovascular anomalies were the leading cause of infant death with a specific infant mortality rate of 0.63 in 1000 live births, in the United States[6].

Prenatal diagnosis of congenital heart defects is still unfulfilling and insufficiently performed with detection rates between 13.7 and 43.8% in low-risk population[7-13]. Prenatal detection is even less effective at lower gestational ages[14] and more efficient if performed by a specialist in fetal echocardiography[15].

Prenatal diagnosis improves the outcome of fetuses with certain types of cardiac anomalies with programmed delivery in tertiary centers[16, 17]. In cases of complex and severe malformations it offers the opportunity for counseling and timely termination of pregnancy.

Few studies have evaluated cardiac anomalies in fetal postmortem examinations[18-20].

The main objective of this study was to evaluate the spectrum of cardiac findings in our fetal autopsy population and to establish the correlation between prenatal and postmortem diagnosis. Secondary aims were the determination of cardiac pathology rate and to provide an analysis of associated extra-cardiac malformations and chromosomal abnormalities.

Material and Methods

A retrospective study of fetal autopsies was performed between 1/1/2002 and 30/06/2010, in a tertiary referral hospital.

Fetal postmortem examinations reports were reviewed, and all cases with cardiac pathology, irrespective of the main cause of death, were selected for analysis.

Termination of pregnancy was performed according to a pharmacological protocol. After 22 weeks of gestation, feticide under fetal analgesia was systematically adopted. Initially we performed feticide with intra-cardiac injection of KCl, but since 2009 we use lidocaine (injected either in the umbilical vein or intra-cardiac).

Parental consent was always obtained before autopsy.

All cases were submitted to postmortem examination at the Developmental Perinatal Unit of the Department of Pathology. A team of a pathologist and two technicians with special training and totally dedicated to perinatal pathology performed all the fetal autopsies and placental examination, according to standardized procedures. The protocol for autopsy has been adapted, modified and improved, over time, according to clinical requirements[21-26]. The malformations, in general, are checked in situ, while the anatomic relationships are preserved. This is extremely important when dealing with congenital heart disease[27]. In the presence of an heart malformation, we proceed with a more detailed dissection, after some hours of fixation using the sequential segmental approach[28, 29]. Photographs are taken at the same time for documentation. Finally, sections are taken, routinely, at the time of the gross necropsic examination, for histological study.

Gestational age was calculated by the crown-rump length measurement in the first trimester ultrasound, and confirmed at autopsy by evaluating several biometric parameters, as well by estimation of organ maturation.

Cytogenetic analysis was performed in all cases. We performed karyotype in fetal cells obtained by amniocentesis or cordocentesis in cases of prenatal diagnosis. In cases of spontaneous fetal loss karyotype was performed in fetal tissue.

Others studies were undertaken whenever necessary, namely polymerase chain reaction (PCR) for viral genome identification.

Three observers (an obstetrician, a pathologist and a pediatric cardiologist) performed the reevaluation of all cases based on reports and photographs.

In order to analyze the type and associations of cardiac pathology, we classified the situs as normal or abnormal. The situs was not evaluated in three cases: ectopia cordis and thoracopagus. Seven main categories were established, based in commonly accepted terminology and classifications, listed in table 2[19, 30, 31]: left ventricular outflow tract obstruction (LVOTO), right ventricular outflow tract obstruction (RVOTO), septal defects, connection anomaly, conotruncal anomaly, complex and others. Each case was assigned to a single category. Complex anomalies were considered whenever anomalies from two or more categories were identified in the same heart. Others anomalies included congenital arrhythmias, myocardium disease, vascular anomaly, position anomaly or thoracophagus. We also classified cardiac defects as isolated or with associated anomalies, whenever there was a chromosomal or a morphological extra-cardiac anomaly.

In the cases with prenatal diagnosis of cardiac anomaly, we performed a correlation between prenatal diagnosis and postmortem findings. The ultrasound and echocardiography reports were reviewed. In

terms of cardiac anomaly, we classified the cases as having complete agreement, complete disagreement and major agreement with additional information[32].
The study was approved by our Hospital Ethics Committee.
Statistical analysis was performed with SPSS Statistics 17.0 (SPSS Inc, Chicago, Illinois). Continuous variables with skewed distribution were reported by median and range (minimum and maximum). The Mann-Whitney U test was used to evaluate the significance of differences between groups in continuous variables with skewed distribution. The χ^2-test or Fisher's exact test was used to determine the two-tailed statistical significance of differences between proportions in 2×2 tables (categorical variables). For all tests, a p-value of less than 0.05 was considered significant.

Results

During the study period a total of 726 fetal postmortem examinations were performed in our Hospital. Abnormal cardiac findings were identified in 99 cases (13.6%): 21 cases resulted from spontaneous fetal demise (4.7%) and 78 cases were submitted to termination of pregnancy (28.2%) (Table 1). Out of the 21 cases of fetal demise, 14 (4.3%) were spontaneous abortions and seven (5.7%) were stillbirths. Considering the cases of termination of pregnancy (n=78), 32 cases (41.0%) were submitted to termination due to a cardiac anomaly, 34 cases (43.6%) due to chromosomal abnormalities, 11 cases (14.1%) due to extra-cardiac malformations, and one case (1.3%) due to rupture of membranes at previable period.

Table 1: Abnormal cardiac findings in 726 postmortem examinations

Cause of death	Postmortem examinations n	Cases with cardiac anomalies n (%)
Fetal demise	449	21 (4.7)
Spontaneous abortion	327	14 (4.3)
Stillbirth	122	7(5.7)
Termination of pregnancy	277	78 (28.2)
Total	726	99 (13.6)

Prenatal diagnosis of cardiac anomaly was done in 50 cases (50.5%) and echocardiogram was performed by a specialist in fetal echocardiography in 41 cases (41.4%).
The median maternal age was 32 years (range 15-43 years).
The median gestational age at the time of fetal demise or termination of pregnancy was 20 weeks (range 11-36 weeks). There was no difference between gestational age at the time of fetal demise (median of 19 weeks, range 11-36 weeks) and gestational age at the time of termination of pregnancy (median of 20.5 weeks, range 12-34 weeks) (p=0.870).
The fetal weight ranged from 3.39 to 3295 g, with a median of 347 g (n=97).
Of the 99 cases evaluated, 54 (54.5%) were male, 44 (44.4%) were female and one case had genital ambiguity (this fetus had a normal male karyotype).

The situs was evaluated in 96 cases and there was situs anomaly in four cases (4.2%): three cases of situs ambiguous (two with right isomerism and one with left isomerism) and one case with situs inversus totalis. All cases with situs anomaly also had a complex anomaly.

The distribution of cases by the seven main categories is shown in table 2. The two most common categories were septal defects and complex anomalies, both occurring in 21 cases (21.2%).

Table 2: Distribution of congenital heart defects by seven main categories and classification as isolated or with associated anomalies

Heart defect category	Total n (%)	**Isolated** n	**Associated anomalies** n	p
Left ventricular outflow tract obstruction	15 (15.2)	7	8	0.235 †
Right ventricular outflow tract obstruction	14 (14.1)	7	7	0.137 †
Septal defects	21 (21.2)	2	19	**0.012** *
Connection anomaly	3 (3.0)	0	3	0.549 †
Conotruncal anomaly	11 (11.1)	5	6	0.327 †
Complex anomalies	21 (21.2)	7	14	0.911 *
Others anomalies	14 (14.1)	4	10	1.000 *
Total	99	32	67	

*χ^2-test; †Fisher's exact

Cardiac anomaly was an isolated finding in 32 fetuses (32.3%) and associated anomalies were found in 67 fetuses (67.7%) (Table 2). A chromosomal abnormality was detected in 42 fetuses and extra-cardiac morphological anomalies were found in 25 fetuses (major anomalies in 22 fetuses and minor anomalies in three). Associated anomalies are described in table 3.

Table 3: Associated anomalies in fetuses with cardiac anomalies

Associated anomaly	n
Chromosomal abnormalities	**42**
Trisomy 21	14
Trisomy 18	13
Trisomy 13	5
Trisomy 9	2
Monosomy X	1
Triploidy	4
Structural anomaly	3
46,XX,del(18)p(11.2)	
46,XX,der(5) t(5,7)(15.1;13)mat	
46,XY,+21,der(21;21)(q10;q10)	
Extra-cardiac structural anomalies	**25**
Renal anomaly (agenesia or dysplasia)	3
Conjoined twins	1
Gastroschisis	1
Growth restriction	1
Pentalogy of Cantrell	2
Hemifacial microsomia (OMIM 164210)	2
Cloacal dysgenesis	3
Situs ambiguus	3
Situs inversus	1
Myelomeningocele + renal dysplasia	1
Omphalocele + renal dysplasia	1
Holoprosencephaly + growth restriction	1
Macrosomia + organomegaly + hydrops	1
Anemia + hydrops	1
Multiple minor anomalies	3
	67

Considering the types of cardiac anomalies (Table 4), ventricular septal defects were the most commonly identified (14 cases), including nine perimembranous defects and two muscular defects. The following heart defects were also diagnosed, in order of frequency: hypoplastic left heart syndrome (9 cases) and atrioventricular septal defects (7 cases: four with complete defects and three with partial defects).

Table 4: Specification of types of cardiac anomalies according to the seven main categories

	n (%)
Left ventricular outflow tract obstruction (LVOTO)	**15 (15.2)**
Hypoplastic left heart syndrome (HLHS)	9
Interrupted aortic arch (IAA)	2
Coarctation of aorta with intact ventricular septum (COA-IVS)	2
Aortic stenosis (AS)	1
Subaortic stenosis	1
Right ventricular outflow tract obstruction (RVOTO)	**14 (14.1)**
Pulmonary valve stenosis (PVS)	4
Tricuspid atresia (TA)	1
Ebstein malformation or anomaly	3
Pulmonary atresia (PA)	2
Tricuspid atresia + pulmonary atresia	4
Septal defects	**21 (21.2)**
Atrioventricular septal defects (AVSD)	7
Ventricular septal defect (VSD)	14
Connection anomaly	**3 (3.0)**
Transposition of the great arteries (TGA)	2
Anomalous pulmonary venous return (APRV)	1
Conotruncal anomaly	**11 (11.1)**
Truncus arteriosus	2
Tetralogy of Fallot (TOF)	7
Double outlet right ventricle (DORV)	2
Complex anomalies	**21 (21.2)**
Others anomalies	**13 (13.1)**
Congenital arrhythmia	1
Supraventricular tachycardia with hydrops	
Myocardial pathology	6
Non-compaction of myocardium	3
Myocarditis	2
Hypertrophic cardiomyopathy	1
Vascular anomaly	4
Right aortic arch	
Anomaly of position	2
Ectopia cordis	
Thoracopagus	1

Twenty one cases were included in the category of complex anomalies and are described in table 5. In this group, seven cases had only the cardiac anomaly and 14 had associated anomalies: five cases had chromosomal abnormalities and nine cases had morphological extra-cardiac anomalies.

In the category of others anomalies (Table 4) there were six cases of myocardial pathology, including three cases of non-compacted myocardium, two cases of myocarditis (one caused with herpes 6) and one case of hypertrophic cardiomyopathy.

The only category of anomalies in which a significant difference was detected between cases with isolated cardiac anomaly and cases with associated anomalies was septal defects, which was more frequent in cases with associated anomalies than in cases of isolated anomalies (p=0.012) (Table 2).
Septal defects was the most common category found in fetuses with a chromosomal abnormality, occurring in 17 fetuses (40.5%). Nine fetuses with septal defects had Down syndrome, five with atrioventricular septal defects and four with ventricular septal defects. LVOTO (6 cases) and RVOTO (6 cases) were the second most frequent categories. Connection anomalies was the only category not represented in cases with chromosomal abnormalities.

Table 5: Discrimination of cases classified in the category of complex anomalies (n=21)

Case	Complex cardiac anomalies	Associated anomaly
26	Left isomerism, complete AVSD, DOLV, non-compaction of myocardium, complete atrioventricular block	Situs ambiguous (left isomerism)
27	Single ventricle, AVSD, DISV, PA	Omphalocele + renal dysplasia
30	TGA, VSD, LVOTO (PS) (double discordance)	Situs inversus totalis
33	Single morphologically left ventricle, DOLV, TA	-
34	Single morphologically left ventricle, DOLV, MA, PVS	46,XX,del(18)p(11.2)
37	TGA, VSD, LVOTO (PA)	-
38	TGA, VSD, TA, APVR (double discordance)	Multiple minor anomalies
42	Ebstein malformation, HLHS, VSD	47,XX,+18
46	Right isomerism, AVSD, PA	Situs ambiguous (right isomerism)
48	PVS, COA	46,XX,der(5) t(5,7)(15.1;13)mat
52	Right isomerism, Single morphologically left ventricle, PA	Situs ambiguous (right isomerism)
53	Complete AVSD, IAA	69,XXX
57	Complete AVSD, IAA	-
58	DORV, IAA	-
59	TGA, VSD, RVOTO (AS), IAA	-
64	AVSD, TOF	47,XY,+21
66	LVOTO (MS), DORV, RVOTO (PS), APVR	Hemifacial microsomia
69	AVSD, Truncus arteriosus, APVR	Hemifacial microsomia
74	AVSD, DORV, PS	Multiple minor anomalies
91	AVSD, LVOTO (AS, IAA)	-
96	AVSD, LVOTO (IAA, COA)	-

AVSD: atrioventricular septal defect, DOLV: double outlet left ventricle, DISV: double inlet single ventricle, PA: pulmonary atresia, TGA: transposition of the great arteries, VSD: ventricular septal defect, LVOTO: left ventricular outflow tract obstruction, PS: pulmonary stenosis, TA: tricuspid atresia, MA: mitral atresia, PVS: pulmonary valve stenosis, APVR: anomalous pulmonary venous return, HLHS: hypoplastic left heart syndrome, COA: coarctation of aorta, IAA: interrupted aortic arch, DORV: double outlet right ventricle, RVOTO: right ventricular outflow tract obstruction, AS: aortic stenosis, TOF: tetralogy of Fallot

In the cases with morphological extra-cardiac anomalies the most frequent categories of cardiac anomalies were complex anomalies (9 cases) and others anomalies (7 cases).
In four cases fetal hydrops was recorded as a consequence of the cardiac anomaly: one hypoplastic left heart syndrome, one tricuspid atresia with pulmonary atresia, one supraventricular tachycardia and one complex cardiac anomaly with complete atrioventricular block (case 26, table 5).
There was no significant difference between gender and categories of anomalies (p=0.822) or between gender and presence of associated anomalies (p=0.737).

The correlation between prenatal and postmortem cardiac findings was performed in the 50 cases with prenatal diagnosis of cardiac anomaly. There was complete agreement in 36 cases (72.0%), major agreement with additional information in ten cases (20.0%) and complete disagreement in four cases (8.0%).

In ten cases (20.0%), the concordance was classified as major agreement with additional information, because the necropsic examination showed additional cardiac anomalies that had no prenatal diagnosis (Table 6). Two cases with prenatal diagnosis of LVOTO at 22-23 weeks had postmortem diagnosis of myocarditis; in one of them, human herpes virus 6 was identified by PCR. In one case with prenatal diagnosis of tricuspid and pulmonary atresia, the necropsic examination displayed a dysplastic pulmonary valve with a mural thrombus, that had conditioned functional atresia.

Table 6: Cases with major agreement and additional information (n=10)

Case	Echocardiogram	Prenatal diagnosis	Outcome	Additional information
16	Yes	APVR total, PS	FD	APVR partial
26	Yes	AVSD, infiltrative CMP	TOP	Left isomerism, AVSD, DOLV, non-compaction
33	Yes	Tricuspid and pulmonary atresia	TOP	SMLV, DOLV, TA
42	Yes	AVSD, Subaortic stenosis	TOP	Ebstein malformation, HLHS, VSD
51	Yes	RVOTO (PA)	TOP	Non-compaction of myocardium
52	Yes	SMLV, PA	TOP	Right isomerism, SMLV, PA
68	Yes	Tricuspid and pulmonary atresia	TOP	Pulmonary dysplasia with thrombus
69	Yes	AVSD, aortic stenosis	TOP	AVSD, Truncus arteriosus, APVR
78	Yes	LVOTO (HLHS)	TOP	Myocarditis
89	Yes	LVOTO (HLHS)	TOP	Myocarditis (herpes 6)

FD: fetal death, TOP: termination of pregnancy, APVR: anomalous pulmonary venous return, PS: pulmonary stenosis, AVSD: atrioventricular septal defect, CMP: cardiomyopathy, DOLV: double outlet left ventricle, SMLV: single morphologically left ventricle, TA: tricuspid atresia, HLHS: hypoplastic left heart syndrome, VSD: ventricular septal defect, RVOTO: right ventricular outflow tract obstruction, PA: pulmonary atresia, LVOTO: left ventricular outflow tract obstruction

In four cases (8.0%), the concordance was classified as complete disagreement, because the cardiac anomaly with prenatal diagnosis was not confirmed at necropsy. But in all of these four cases, another cardiac anomaly was identified (Table 7).

Table 7 : Cases with complete disagreement (n=4)

Case	Echocardiogram	Prenatal diagnosis	Outcome	Additional information
17	Yes	AVSD	FD	LVOTO (HLHS)
43	No	AVSD	TOP	LVOTO (HLHS, IAA)
44	No	VSD	TOP	LVOTO (IAA)
96	No	LVOTO (HLHS)	TOP	AVSD, LVOTO (IAA, COA)

FD: fetal death, TOP: termination of pregnancy, AVSD: atrioventricular septal defect, LVOTO: left ventricular outflow tract obstruction, HLHS: hypoplastic left heart syndrome, IAA: interrupted aortic arch, VSD: ventricular septal defect, COA: coarctation of aorta

Fetal echocardiogram performed by a specialist was undertaken in 41 cases (82.0% of cases with prenatal diagnosis). In this group there was complete agreement in 30 cases (73.2%), major agreement with additional information in ten cases (24.4%) and complete disagreement in one case (2.4%). In the nine

cases with prenatal diagnosis of cardiac anomaly without specialized echocardiogram there was complete agreement in six (66.7%) and complete disagreement in three (33.3%). Whenever the echocardiogram was not performed by a specialist the number of cases classified with complete disagreement was superior (33.3% vs 2.4%) (p=0.012).

Discussion

The prevalence of cardiac defects in our autopsy population (13.6%) is higher than that found in epidemiological studies undertaken in live-births[5, 33, 34]. However it is similar to that found in series of fetal or perinatal autopsy[18, 19]. The high prevalence of cardiac defects in our series is probably due to the nature of the population studied (a series of fetal autopsy cases), with an elevated proportion of termination of pregnancy. Moreover, our hospital is a referral centre for congenital cardiac pathology which explains the higher number of cases submitted to termination of pregnancy specifically due to cardiac defects (11.5%)[19, 35, 36].

In our study, 67 cases (67.7%) had associated anomalies, either chromosomal abnormalities or structural extra-cardiac anomalies, which are higher than that found by Song and colleagues in theirs fetal series (36.9%)[37] and by Meberg and colleagues in theirs neonatal series (22%)[34]. Mitchell *et al.* in a birth cohort (n=46109) reported associated anomalies in 46% of cases of stillbirths and neonatal deaths with congenital heart defects[5] and Grant and Evans reported associated anomalies in 78% of cases of fetal and pediatric autopsies[19]. We found associated anomalies in all heart defect categories in good accordance with others[19]. The proportion of cases with chromosomal abnormalities in fetuses with cardiac anomalies (42.4%) is similar to that found by Paladini *et al.* (48%)[38] and higher than that described by Tennstedt *et al.* (33%)[18] and by Song *et al.* (19.1%)[37].

The commonest cardiac anomaly was ventricular septal defect, in agreement with others studies[5, 18, 20, 34]. However, in our series, it represents a lower proportion (14.1%), which is probably due to the greater proportion of complex anomalies (21.2%). We classified as complex 21.2% anomalies, higher than that found by Grant and Evans (4.0%)[19].

In cases with chromosomal abnormalities, the most frequent category of cardiac defect found was septal defects, in spite of almost all kinds of categories (except connection anomalies) have been detected, and not just the expected anomalies. In a prospective study of 1006 fetuses with congenital heart disease, chromosomal anomalies were not found only in cases with transposition, critical aortic stenosis and double-inlet ventricles[39]. In Down syndrome, septal defects were the most frequent category of cardiac defect (64.3%), in agreement with other reports[40-45]. However, we also had one complex anomaly in a fetus with Down syndrome[46].

Additional sampling of the heart for histology was essential for the identification of myocarditis and non-compacted myocardium. We identified HHV-6 genome in paraffin embedded heart tissue in one case with lesions of myocarditis in the histology[47].

The higher proportion of males in our population of fetuses with cardiac anomalies is in good agreement with others studies[18, 48]. However, when we analyzed them by categories, gender predominance has not been identified[49], probably due to the small size of the sample.

The correlation between prenatal and postmortem findings concerning cardiac anomalies demonstrated complete agreement in 36 cases (72%). Excluding the cases with complete disagreement (4 cases, 8%) the

agreement rate increased to 92%. Despite the different study methodology, our results are similar to others[20, 50, 51].

When the prenatal evaluation was performed by a pediatric cardiologist specialized in fetal echocardiogram the agreement between prenatal and postnatal findings was higher. This is consistent with others studies that have demonstrated that the prenatal detection of heart defects is improved if performed by a specialist in fetal echocardiography[15, 52, 53].

The high prevalence of cardiac defects, some of them lacking prenatal diagnosis, highlights the importance of examining the heart in all cases of fetal death or termination of pregnancy. Additional sampling of the heart for histology is also essential. This paper stresses the importance of developmental pathology, obtaining information as much as possible, in order to optimize the diagnosis, audit the quality of prenatal diagnosis, and to estimate the recurrence risk to improve parental counseling.

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6.2 Human herpes virus 6 causing myocarditis in a fetus: an infectious cause to a congenital heart defect

Ramalho C, Monterroso J, Simões J, Brandão O, Montenegro N. Human herpes virus 6 causing myocarditis in a fetus: an infectious cause to a congenital heart defect. J Clin Pathol 2011; 64: 733-4

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CORRESPONDENCE

Human herpes virus 6 causing myocarditis in a fetus: an infectious cause to a congenital heart defect

We read with interest the article published by Comar *et al*. The authors investigated the presence of human herpes virus 6 (HHV-6) genome in archival explanted myocardial tissue of children who underwent heart transplantation due to cardiomyopathy or congenital heart disease. HHV-6 genome was detected in 11 of the 35 (31.4%) hearts. The authors raise the question of the significance of HHV-6 in heart disease. Does HHV-6 play a role in the development of cardiomyopathy or congenital heart defects?[1]

We report a case of fetal myocarditis in which a PCR study of cardiac tissue showed the presence of HHV-6. To the best of our knowledge, this is the first case of HHV-6 infection *in utero* associated with myocarditis and congenital heart abnormality.

A 15-year-old primigravida at 22 weeks gestation was referred to our Hospital due to fetal dilated cardiomyopathy. Her past medical history was irrelevant without febrile illness or suspected viral infection during pregnancy. The fetal echocardiography showed enlargement of the right atrium, left ventricle and right ventricle. The contractility was reduced in the right ventricle and absent in the left ventricle. There was also mitral and aortic stenosis.

After counselling, the pregnant woman and her mother decided to terminate the pregnancy. Cordocentesis was performed and the fetal karyotype obtained from the fetal blood was 46, XY. The pregnancy was terminated by injecting lidocaine in the umbilical cord.

Necropsy examination showed a 582 g male fetus with biometry and maturation concordant with a gestational age of 23 weeks. The heart was dysmorphic with dilation of ventricles, especially on the left. The tricuspid valve was dilated with thickened septal cusp. The mitral valve was abnormal with commissural fusion and fibrous thickened cusps and cords (figure 1A). The pulmonary valve was normal and the aortic valve was stenotic (figure 1B).

A detailed microscopic examination showed infiltration of mononuclear cells and sparse neutrophils in the interstitium of the myocardium (in the septum, left and right ventricles), together with multiple areas of myocytolysis and disorganisation of myocytes corresponding to a diagnosis of myocarditis with activity (figure 2A, B). There was fibrosis in the sub-endocardial location with foci of calcification in both ventricles, more extensively in the left ventricle and in the mitral cusps, as well as in the subvalvular apparatus (figure 2C, D). Calcification was also identified in left ventricle papillary muscles. There were also small foci of necrosis in the right ventricle.

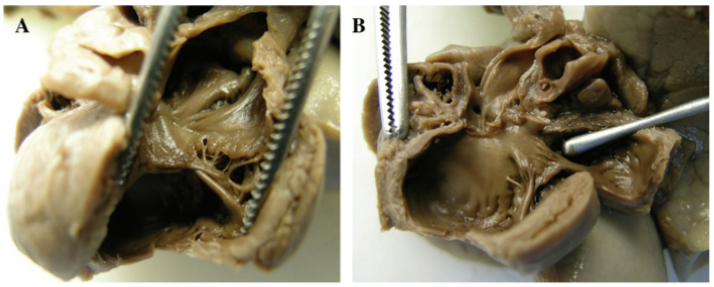


Figure 1 Macroscopy. (A) Mitral valve with severe deformation of subvalvular apparatus. (B) Stenotic aortic valve with dysplastic cusps. Dilated and deformed cavity coated by fibrous tissue with calcification and without trabeculation; absent apex.

Except for cardiac anomaly, no other dysmorphic or inflammatory features were identified in other organs.

Following maternal serological results, PCR techniques were used to determine the presence of viral genome in fetal blood (enterovirus, rubeola virus, cytomegalovirus and adenovirus) and tested negative. Then PCR techniques were used to look for viral genome (Epstein–Barr virus, Herpes simplex 1, Herpes simplex 2, HHV-6 and HHV-7) in formalin-fixed tissues (myocardium and mitral valve). Only HHV-6 DNA was detected.

Despite congenital infection with HHV-6 having an established prevalence of 1%,[2] there are few reports associating HHV-6 to disease in fetuses. Congenital HHV-6 infection has been considered asymptomatic, even in the presence of active replication.[2] Ashshi *et al* report two fetuses with hydrops in which HHV-6 DNA was detected, suggesting a possible role of HHV-6 in fetal hydrops.[3] But both positive cases had chromosomal abnormalities, a recognised cause of fetal hydrops. The authors had suggested that possibly HHV-6 was an additional factor contributing to fetal hydrops.

Recently, HHV-6 has been implicated in myocarditis in adults and children[4–8] but there was no previous association with myocarditis in fetuses.

The cellular receptor for HHV-6 was recently identified as CD46, a ubiquitous protein expressed on the surface of different types of human cells. Since CD46 is expressed on vascular endothelial cells and on muscle cells, HHV-6 infection and virus-induced pathology are possible in the heart.

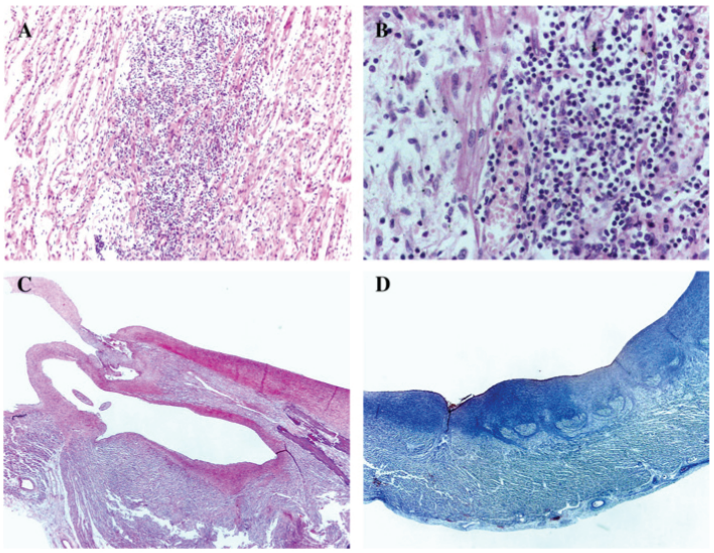


Figure 2 Histological examination. (A, B) Myocarditis (HE stain; A: 100×; B: higher magnification). (C) Mitral valve with fibrosis and calcification (HE stain, 20×). (D) Left ventricle wall with sub-endocardial and interstitial fibrosis (Masson's trichrome stain, 20×).

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Take-home messages

- Despite congenital infection with HHV-6 having an established prevalence of 1%, there are few reports associating HHV-6 to disease in fetuses.
- HHV-6 has been implicated in myocarditis in adults and children, but there was no previous association with myocarditis in fetuses.
- The virus-induced myocardial injury and the subsequent reparative lesions in the heart in development may be the cause of structural cardiac defects in fetuses.

We consider that in our case, the presence of HHV-6 DNA in the myocardium, in the absence of other pathogens, suggests that the myocarditis was caused by the virus. Moreover, we suggest that the identification of the viral genome is favourable to a pathogenic relationship between the virus and the cardiac anomaly.

In our case, there are pathological findings of acute, subacute and chronic myocarditis compatible with viral persistence. The infection probably occurred early in the second trimester of pregnancy. We postulate that the virus-induced myocardial injury and the subsequent reparative lesions in the heart in development may be the cause of structural cardiac defects in this fetus.

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Progressive macular hypomelanosis Guillet-Hélénon: structural and immunohistochemical findings

In 1985 and 1988, Gerard Guillet and Raymond Hélénon, with colleagues, described a novel acquired hypopigmentation disorder in dark-skinned immigrants from the Caribbean which they named progressive macular hypomelanosis (PMH).[1 2] Over the past 20 years, many other terms have been coined for this condition, including cutis trunci variata, nummular and confluent hypomelanosis, Creole dyschromia, or idiopathic multiple large-macule hypomelanosis.[3] More than 600 PMH patients have been reported in the literature with skin phototypes II–VI, but the debate about the aetiology of this disorder is still open.

We present an immunohistochemical and ultrastructural study on disseminated PMH extending beyond the trunk. No *Propionibacterium* colonisation of skin was found despite extensive examination. The evidence for a bacterial origin is discussed in consideration of the literature.

A 29-year-old Romanian female patient (skin phototype III), who had relocated to Germany 8 years earlier, presented with a mottled hypopigmentation of her skin that had progressed over the past 12 years. At the age of 17, macules started to appear on the abdomen and then spread gradually to the flanks, axillae and back. Over the past 2 years, the patient had noticed a rapid progression to the neck, décolleté and groin without any subjective symptoms. Physical examination revealed multiple partially coalescing oval or irregular hypopigmented macules ranging in diameter from 1 to 5 cm without any scaling (figure 1A). There was no fluorescence under illumination with Wood's light (figure 1B). Other family members were not affected.

Blood tests including HIV-ELISA were unremarkable with the exception of TPPA (1:160). Standard bacterial cultures (McConkey-sheep blood agar, 37°C) from skin swabs revealed coagulase-negative *Staphylococcus aureus*. Cultures under microaerophilic and dark conditions on brain–heart infusion agar (pH 7.1, 37°C) were negative for *Propionibacterium* species. *Treponema pallidum* DNA was not found after PCR in PMH lesion.

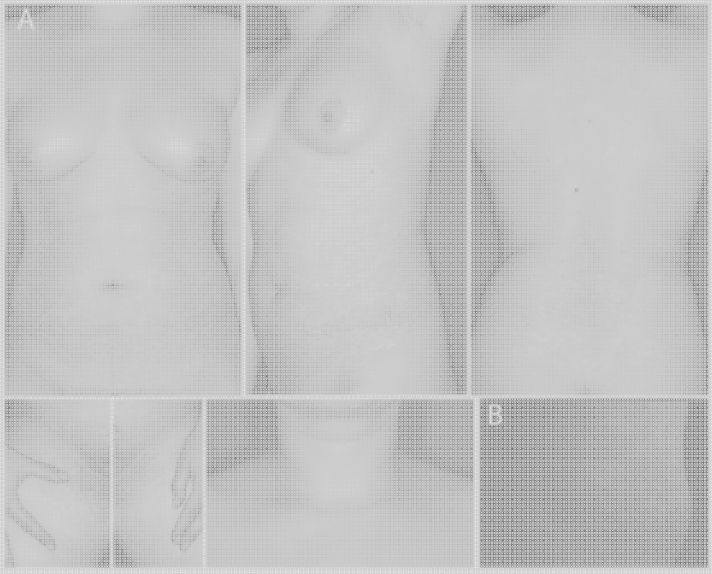


Figure 1 (A) Disseminated progressive macular hypomelanosis in a 29-year-old female patient. (B) No follicular fluorescence under Wood's light (UVA 320–400 nm), right flank part.

Capítulo 7

Discussão e conclusões

7.1 Discussão geral

O principal objectivo deste trabalho foi a correlação entre o diagnóstico pré-natal e o diagnóstico *post-mortem* com vista à melhoria da acuidade do diagnóstico pré-natal. Para se conseguir alcançar este objectivo foram realizados vários estudos.

No **Capítulo 3** foi efectuada a **correlação entre o diagnóstico pré-natal e o diagnóstico *post-mortem* nas situações de interrupção médica da gravidez de causa fetal.**

No estudo realizado (**Capítulo 3.1**) foram avaliados 76 casos de interrupção da gravidez de causa fetal. As anomalias morfológicas constituíram o principal motivo para a interrupção (47%) seguidas pelas alterações cromossómicas (33%), dados estes semelhantes aos de outros estudos[1].

No nosso estudo as interrupções tardias (depois das 24 semanas) representam 8,3%, número que é inferior ao que ocorre noutros países, nomeadamente em França (32,9%)[1] e Israel (29%)[2].

A comparação entre os achados pré-natais e os achados *post-mortem* mostrou uma concordância total em 61,1% dos casos, similar aos valores encontrados noutros estudos, com valores entre os 42% e os 72,5%[3-9]. Não houve nenhum caso de discordância total, ao contrário do encontrado por outros autores, com discordância entre 0,2 a 2,5%[3-5, 9].

Poderá ser questionada a opção pela avaliação da concordância na totalidade dos casos submetidos a interrupção. O diagnóstico definitivo já está previamente estabelecido nos casos de anomalias cromossómicas, pelo que não deveriam ter sido incluídos na análise. Contudo, a avaliação da concordância, excluindo os casos de anomalias cromossómicas, mostra resultados similares, com uma concordância total em 58,9%[10].

A autópsia permitiu alterar o risco de recorrência em 32% dos casos em que acrescentou informação, de acordo com os resultados de outros trabalhos[4, 11].

Apesar da acrania apresentar um fenótipo característico, pode ter diferentes etiologias, com riscos de recorrência diferentes. Procedemos à avaliação de 14 casos de acrania (**Capítulo 3.2**), dos quais apenas oito representavam uma anomalia isolada. Detectamos anomalias cromossómicas em três dos oito casos analisados, o que é superior ao descrito (0,7-5,6%)[12-16]. Nos casos de trissomia 18, a aneuplodia mais frequentemente associada a defeitos do tubo neural[13, 15], o risco de recorrência é menor. Nas anomalias estruturais, quando a anomalia é herdada, o risco é superior.

A disgenesia tubular renal **(Capítulo 3.3)** é um exemplo de uma doença letal, autossómica recessiva, em que o diagnóstico definitivo só é possível após a realização da autópsia[17]. O diagnóstico de uma doença autossómica recessiva tem um impacto importante, ao estabelecer um risco de recorrência de 25%.

No caso da gravidez gemelar com os dois fetos afectados com hérnia diafragmática (**Capítulo 3.4**), a autópsia detectou a presença de hérnia bilateral, com as mesmas características fenotípicas em ambos os fetos. Este facto suscitou a realização de estudo genético que confirmou tratar-se de uma gravidez monozigótica. Este caso representa mais um dado a favor da implicação de factores genéticos na etiologia da hérnia diafragmática[18, 19].

No **Capítulo 4** foi avaliado o **contributo da autópsia nos casos de interrupção médica da gravidez por anomalias cromossómicas**. Nos casos de interrupção médica da gravidez por anomalias cromossómicas o diagnóstico definitivo já está estabelecido, mas a autópsia pode detectar anomalias não identificadas previamente.

Avaliamos 57 casos de interrupções devidas a anomalias cromossómicas, efectuadas até às 24 semanas, compreendendo 46 trissomias, quatro triploidias, três anomalias estruturais e quatro anomalias dos cromossomas sexuais (**Capítulo 4.1**). Neste grupo há 10,5% de anomalias raras, de acordo com os resultados observados por Nicolaides e colaboradores (10.4%)[20], mas superior ao valor encontrado por Baena e colaboradores (6.6%)[21].

A autópsia forneceu informação adicional em 26,3% dos casos. Foram detectadas anomalias major adicionais em 12,2% dos casos e em 14% uma anomalia detectada na ecografia não foi confirmada. O grupo de anomalias em que se observou maior acréscimo de informação foi o grupo das anomalias cardíacas, de acordo com outros trabalhos[22]. Em cinco casos foi identificada uma anomalia cardíaca e noutros cinco não foi confirmada a presença de anomalia cardíaca. Conhecida a baixa acuidade de diagnóstico destas anomalias[23-29] e porque a presença de anomalias associadas pode ser determinante para a decisão dos casais[30-33], deve efectuar-se ecocardiograma antes dos casais decidirem pela interrupção de gravidez.

Descrevemos em pormenor dois casos de trissomia 9 (**Capítulo 4.2**) e um caso de trissomia 12 (**Capítulo 4.3**), em que o exame necrópsico detectou múltiplas anomalias, o que contribuiu para a caracterização morfológica destas aneuploidias raras.

No terceiro estudo (**Capítulo 5**) procedeu-se à avaliação da **variabilidade fenotípica na síndrome de Down**. Apesar do fenótipo da síndrome de Down poder ser, em parte, reconhecido a partir das 14 semanas[34], um feto do 2º trimestre pode ainda não ter definidas as alterações características da face e cabeça, parecendo normal mesmo a observadores experientes[35].

No estudo caso-controlo efectuado, compreendendo 50 fetos com síndrome de Down e 47 controlos, os parâmetros biométricos que apresentavam diferenças com significado estatístico foram apenas o diâmetro occipito-frontal e o comprimento do osso do nariz. A razão BPD/OFD é maior nos fetos com síndrome de Down o que confirma que a braquicefalia é uma característica precoce nestes fetos[36-38]. Uma alteração detectada também no 2ª trimestre é a diminuição do crescimento do cérebro[37].

O comprimento do osso do nariz é significantemente menor nos fetos com síndrome de Down, de acordo com estudos radiológicos *post-mortem*[36, 39-41], apesar de no nosso estudo encontrarmos uma prevalência superior de fetos normais com ossos ausentes ou hipoplásicos[42].

De acordo com outros estudos[43, 44] encontramos anomalias craniofaciais em 96% dos fetos com síndrome de Down. Quando avaliamos as anomalias craniofaciais isoladamente, encontramos diferenças com significado estatístico em todas excepto no tamanho das orelhas. O número de casos avaliados pode não ter sido suficiente para encontrar diferenças no número de fetos com orelhas pequenas, devido à pequena diferença no tamanho das orelhas dos fetos[45-47].

Um parâmetro em que as diferenças encontradas foram superiores às previamente descritas foi o edema da nuca[44], podendo explicar-se pela maior subjectividade da avaliação necrópsica, em comparação com a avaliação ecográfica.

Apesar da dificuldade de comparação, a presença de anomalias do hábito interno em 62% dos fetos com trissomia 21, muito superior ao descrito em estudos epidemiológicos (35.6% e 46,8%)[48, 49] pode dever-se à letalidade inerente à cromossomopatia.
As anomalias cardíacas estão presentes em 38,8% dos fetos, de acordo com os dados de vários estudos (42-44,6%)[43, 49, 50], mas inferior (53-55.9%)[51, 52] e superior (23,4-26%)[44, 53] aos valores de outros.

No **Capítulo 6** avaliamos o **contributo da autópsia perinatal nas anomalias cardíacas congénitas**. A prevalência de anomalias cardíacas (13,6%) no grupo de fetos estudados (**Capítulo 6.1**) é superior à encontrada em estudos epidemiológicos efectuados em nados-vivos[54-56], mas é semelhante à encontrada em séries de autópsia fetal ou perinatal[22, 57]. A elevada prevalência é explicada pelas características da população estudada, uma série de casos de autópsia fetal com uma proporção elevada (11.5%) de casos submetidos a interrupção da gravidez por anomalia cardíaca[3, 4, 57].
Nos 99 fetos com anomalias cardíacas detectamos 67,7% de casos com anomalias associadas, quer cromossómicas quer estruturais extra-cardíacas. A prevalência de anomalias associadas (22%-78%) encontrada nos vários estudos publicados tem uma grande variabilidade[55-58], em parte devida às diferenças metodológicas.
A anomalia cardíaca mais frequente foi o defeito do septo ventricular, de acordo com outros estudos[22, 55, 56, 59], apesar de numa proporção menor, devido à prevalência maior de anomalias complexas[57].
A avaliação da concordância entre os achados pré-natais e *post-mortem* no que respeita às anomalias cardíacas mostrou uma discordância total em 8% dos casos, de acordo com outros trabalhos[59-61]. Quando a avaliação pré-natal foi efectuada por um cardiologista pediátrico a discordância foi menor (2.4% vs 33.3%) (p=0.012)[62-64].
Em alguns casos de anomalias cardíacas, o exame necrópsico possibilitou o diagnóstico etiológico. No caso descrito (**Capítulo 6.2**), a identificação de genoma vírico no miocárdio, associada a miocardite, permitiu, pela primeira vez, estabelecer a relação patogénica entre a infecção por HHV-6 e a anomalia cardíaca[65].

De ressaltar que um dos pontos fortes do nosso trabalho foi a realização de autópsia em todos os casos de interrupção médica da gravidez, o que não acontece noutras instituições. Na revisão efectuada por Gordijn e colaboradores a taxa de autópsia nas interrupções de gravidez variou entre 79 e 100%[66]. Em 2004 foram publicados dois trabalhos que referem valores inferiores para a taxa de realização de autópsia em Inglaterra. Em Oxford, a taxa decresceu de 84% para 67%, de 1991-1995 para 1996-2000[4], e em Birmingham, a taxa foi apenas de 30,7%[5]. Em Israel a taxa de realização de autópsia é ainda mais baixa, 11,2%, com valores de 2,1% nas interrupções antes das 23 semanas e de 33 a 42,4% nas interrupções tardias[2, 67].
Uma outra vantagem é o facto da autópsia ter sido efectuada por uma equipa dedicada à patologia do desenvolvimento, e que segue um protocolo rigoroso. Em muitos dos estudos a equipa que efectua a autópsia não é descrita[68-70] ou então ela é efectuada por um ou vários patologistas não diferenciados[71].
Outra vantagem do nosso trabalho foi a preservação dos fetos estudados, em consequência do método utilizado na nossa instituição para a interrupção da gravidez, com a expulsão induzida farmacologicamente.

No entanto, o carácter retrospectivo da análise efectuada pode ser considerada uma limitação do nosso estudo. Todos os casos que avaliamos foram submetidos a interrupção médica após a realização dos exames considerados adequados pelos clínicos da nossa instituição, sem qualquer interferência dos investigadores, o que será uma vantagem quando consideramos a avaliação da qualidade.
Uma outra limitação dos nossos estudos foi o número de casos avaliados, que pode não ter sido suficiente para avaliar anomalias com baixa incidência (Capítulos 3 a 6) bem como pequenas diferenças entre os casos e os controlos (Capítulo 5).

7.2 Conclusões finais

A realização sistemática da autópsia em situações de interrupção médica da gravidez permite, em muitos casos, estabelecer o diagnóstico definitivo e redefinir o prognóstico, contribuindo para um adequado aconselhamento dos casais (Capítulos 3 e 6).

Nos casos das anomalias cromossómicas a autópsia permite detectar anomalias que podem contribuir para uma melhor caracterização morfológica e conhecimento da história natural das aneuploidias em idades gestacionais precoces (Capítulos 4 e 5).

No caso particular da síndrome de Down, um melhor conhecimento do fenótipo fetal pode ajudar a definir novos marcadores ecográficos que contribuam para melhorar a capacidade de identificação pré-natal deste síndrome (Capítulo 5).

A autópsia com avaliação exaustiva do coração é importante para a detecção e melhor caracterização morfológica das anomalias cardíacas (Capítulos 4 e 6).

Os nossos dados confirmam a importância da autópsia para a auditoria e o controlo de qualidade do diagnóstico pré-natal e para a avaliação da acuidade diagnóstica da ecografia pré-natal (Capítulos 3, 4 e 6).

7.3 Referências

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