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BASIC SCIENCES

CLINICAL MEDICINE

MEDICAL EDUCATION

Immunity to human influenza A – an overview

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Abstract

The effector immunological mechanisms that function to prevent, contain and eliminate infection by type A influenza viruses in humans are reviewed. The role of vaccination in eliciting protective immunity against influenza A, and differences between seasonal and pandemic influenza A virus strains that may be responsible for the lower immunity and greater pathogenicity observed in pandemic influenza, are outlined.

Key Words: *immunity, immunopathology, influenza A, vaccination*

Introduction

Influenza viruses belong to the family Orthomyxoviridae and are a common viral cause of human illness. Three main types of influenza viruses, viz A-C, are responsible for disease in humans [1]. Type A also infects domestic and wild animals. There are many subtypes of A due to antigenic variation and therefore it is mainly responsible for the seasonal outbreaks of influenza in winter in temperate climates and possesses the potential to cause devastating pandemics. Type B in contrast causes sporadic outbreaks of mild disease, is mainly restricted to humans, and shows less antigenic variation than Type A. Type C is a common virus that is well adapted to humans and therefore rarely causes disease [1]. The present review focuses on Type A influenza which is of the greatest interest from a human health perspective. Influenza can lead to fatal secondary infections like pneumonia, particularly in immunocompromised persons and in elderly persons where the immune system progressively become less effective.

The Type A virus has eight different negative-sense RNA strands in a virion. Each of the RNA molecules contains one or more genes coding for different viral proteins [2] as shown in Table 1.

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Table 1. Genome and Proteome of the Influenza A Virus

RNA/Gene	Protein	Function
HA	Haemagglutinin (HA). Three different antigenic types viz. H1, H2, and H3, are found in human viruses; At lease 13 others in animal flu viruses	Binding of host cell sialic acid receptors for invasion; fusion of endosomal membrane with viral membrane allowing escape of RNA and viral proteins into the host cell cytoplasm
NA	Neuraminidase (NA). Two antigenic types in human viruses and seven others in animal viruses	Hydrolysis of terminal sialic acid from virions membrane facilitating virion release and its motility in respiratory tract
NP	Nucleoprotein	Structural
M	Matrix proteins M1 and M2	M1- Structural; M2- Proton channel
NS	Non-structural proteins NS1 and NS2 found in host cell cytoplasm but not virions	Suppression of host pre-mRNA processing and innate immunity; Transport of viral ribonucleoprotein particles from nucleus to cytoplasm
Pol 1-3	Three subunits of the viral RNA-dependent RNA polymerase PA, PB1 and PB2 and a pro-apoptotic protein PB1-F2	Viral RNA replication

The influenza virus is an enveloped virus that in transmission electron micrographs shows prominent spikes extending outward from the lipid bilayer (Figure 1). These are due to surface bound neuraminidase (NA) and trimers of haemagglutinin (HA). During the infection process [2], the A-type virus binds to sialic acid residues on the host cell plasma membrane though HA molecules causing the formation of an endosome. The subsequent acidification of the endosome produces a conformation change in the HA that the causes the viral envelope to fuse with the membrane of the endosome, thereby releasing the viral RNA and protein contents of the virion into the host cell cytoplasm. The viral nucleoprotein then translocates to the nucleus where -ve strand RNA is copied to form +ve RNA strands by the

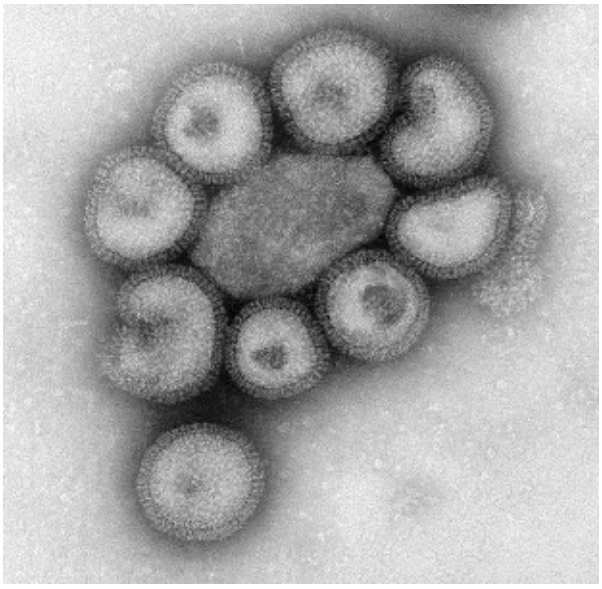

Figure 1. Influenza virus particles**.** The surface spikes are due to Neuraminidase and Haemagglutinin molecules. Courtesy of Center for Disease Control/ Dr. F. A. Murphy [via pingnews] ID#: 8432.

viral RNA polymerase. The +ve strand RNA moves out of the nucleus to serve as mRNA for the synthesis of viral proteins in the endoplasmic reticulum. Glycoproteins like HA and NA become incorporated into the host cell plasma membrane after processing by the endoplasmic reticulum and Golgi body. Extra copies of the –ve strand genome of the virus are also produced by the viral RNA polymerase and together with the relevant proteins are assembled into budding virions that are released from the infected cell with the help of the NA.

HA and NA glycoproteins, are the major target antigens for neutralising antibodies. However HA and NA accumulate mutations in critical epitopes that lead to escape from an anti-viral immune response. Typical winter epidemics involve this *Antigenic or Genetic Drift* which produces new strains of viruses within a subtype.

Antigenic or Genetic Shift results from major changes in antigenicity of HA and NA due to re-assortment with animal and bird influenza viral genomes in co-infected cells. This produces new subtypes of viruses with that differ significantly in antigenicity. The segmented RNA genome of the influenza virus facilitates this process. Such re-assortment can result in a markedly decreased human population immunity to the new strain of virus. In turn, this can lead to epidemics and pandemics, depending on the extent of antigenic difference in the new strain. After the introduction of standardized nomenclature, Influenza A is referred to by the major serotype or subtype e.g. H1N1, and strain e.g. A/Brisbane/2007. A pandemic is usually due to a change in the subtype of HA e.g. the 1968 pandemic involved the emergence of H3N2. The World Health Organization has therefore developed a network of collaborating centres to continuously monitor influenza antigenicity and produce seed material for seasonal vaccine manufacture.

Influenza A pandemics have caused considerable human mortality in the past [1]. The 1918 “Spanish” influenza, due to a H1N1 type virus, caused approximatley 20-50 million deaths worldwide. A 1957 pandemic of H2N2 resulted in 2 million deaths. The lower mortality of this pandemic is attributed to better health care and the availability of antibiotics for the treatment of secondary bacterial infections. In 1968 a pandemic of H3N2 caused 1 million deaths. In 1997, a H5N1influenza A strain that was highly virulent to birds and also able to cause severe illness or death in humans emerged. It was spread by wild birds and poultry, but because of poor human to human transmissibility of the virus no pandemic has resulted to date. In 2009 a new H1N1 strain emerged in Mexico to cause a pandemic. This strain is referred to as 2009 A(H1N1). The pandemic occurred despite the fact that H1N1strains had been circulating in human populations since 1977 and H1N1 (Strain A/ Brisbane/2007) was a component of the 2008/9 seasonal influenza vaccine. The new “swine influenza” variant has been shown to contain many genetic re-assortments with common swine influenza virus genes. Its emergence however has caused a need to rethink the nomenclature of influenza A virus typing, because although typed as H1N1, the antigenicity of its HA was significantly different from the HA of the seasonal H1N1 virus that had been circulating in human populations at that time [3].

Neutralising antibodies that are produced by vaccination or natural infection are largely directed against HA and NA and are therefore generally type and subtype specific. Also there is little immunity against antigenic drift viruses and this results in seasonal influenza epidemics. However there is

data from epidemiological and cotton rat model studies to suggest that a degree of heterosubtypic immunity can develop after natural infection or vaccination [4, 5]

2. Innate Immune Mechanisms in Immunity to Influenza

Infection with influenza virus initially activates the innate immune system through the utilization of specific receptors in human cells termed Pattern Recognition Receptors (PRR). The PRR include Toll-like receptors that recognize viral nucleic acids in endosomes as well as cytoplasmic molecules recognizing unusual structural features of viral RNA. Other viral molecules can activate inflammasomes in macrophages to release inflammatory cytokines like IL-1 and IL-18 [6]. The major mechanisms that play a role in the innate immune response are summarized in Table 2.

The innate immune response provides a measure of protection against infection until the more effective and specific adaptive immune response comes into action

Table 2. Effector Mechanisms in Innate Immunity to Influenza

Induction	Effector cell or molecule	Mechanism of action
-	Naturally occurring mucins	Bind virus
Altered surface of the virion and infected cells	Complement	Activation through the alternate or lectin pathway to promote lysis and opsonisation
PRR	Type 1 interferon (α, β)	Induction of anti-viral state in infected and neighbouring cells through inhibition of protein synthesis and mRNAdegradation. Activation of phagocytic cells and dendritic cells
PRR	Inflammasome in macrophages and dendritic cells	Formation of IL-1, IL-6 and TNFα that promote an inflammatory response in tissue, fever and the synthesis of acute phase proteins
PRR	Macrophage and dendritic cell synthesis of IL-12, IL-18	Activation of NK cells to lyse virus infected cells and enhancement of the adaptive immune response
Stress molecules expressed by infected cells	γδT cells secreting Type 2 interferon γ	Activation of NK cells, phagocytes, dendritic cells and the adaptive immune response

and also has an essential role in activating the adaptive immune response through the supply of relevant cytokines.

3. Adaptive Immune Mechanisms in Immunity to Influenza

protection against infection. Activation of the B cells to become antibody secreting plasma cells takes place in the draining lymph nodes and non - encapsulated mucosa - associated lymphoid tissue in the nasopharynx and the lung. Experiments in animal models show that the appearance of first IgA antibodies and later IgG antibodies in nasal washings correlates well with the decrease in viral titres in the same washings [7]. B cell activation requires helper T cells (T_H cells) that recognize peptides derived from viral proteins that can be membrane, matrix and nucleoproteins, displayed by class II major histocompatibility complex (MHC) molecules on antigen presenting dendritic cells.

Table 3. Effector Mechanisms in Adaptive Immunity to Influenza

Effector Molecule or cell	Mechanism of action
IgA antibodies in mucus and mucosa	Preventing virus binding to host cells through agglutination and neutralization
IgG antibodies in mucus, mucosa and blood	Preventing virus binding to host cells through agglutination and neutralization, activation of complement through the classical pathway, promoting opsonisation and phagocytosis, assisting NK cell killing through Fcγ receptors
T_H Cells	Activation of B cells, promoting immunoglobulin class switching and affinity maturation, secretion of cytokines like IFNγ that activate phagocytes and NK cells and upregulate MHC molecules.
T_c	Apoptosis of virus-infected cells through action of granzyme perforin, etc.

The major adaptive immune mechanisms that serve to prevent infection by limiting viral spread and then eliminating the virus are summarized in Table 3. These mechanisms have been extensively reviewed elsewhere [7].

Dimeric IgA antibodies secreted into the mucus of the respiratory tract provide the first line of defense by the adaptive immune system. IgG antibodies in blood and interstitial fluids have an important role in limiting the systemic spread of the virus. High affinities and levels of these primarily subtype - specific antibodies facilitate

CD8+ Cytotoxic T cells (T_C) are involved in containing and clearing an established infection. Their activation depends on presentation of viral peptides in the context of class I MHC molecules by antigen presenting dendritic cells. This occurs in the draining lymph nodes and mucosal lymphoid

tissues. Activated effector T_C kill virus infected epithelial and other cells displaying viral peptides on the surface in association with Class I MHC molecules. In mouse models, CD8+ cells have been shown to appear in the nasal mucosa 5days post-infection and peak on day 7 [7].

4. Characteristics of the Adaptive Immune Response and Vaccination Against Influenza

Both natural infection and parenteral vaccination produce good protective immunity against the vaccinating strain of virus within a subtype and this phenomenon forms the basis for the seasonal trivalent influenza vaccine that is administered parenterally. The typical influenza vaccine is formalin-inactivated virus produced in eggs. It usually incorporates three major strains in common circulation (two A strains of different subtypes and one B strain) and has a reported efficacy of 60-70%. A vaccine that is reportedly 100% effective is a trivalent cold adapted (and thereby attenuated) live vaccine produced in eggs termed FluMist™ produced by MedImmune [1]. This vaccine is nasally administered as a spray but only to 2-49y old persons who have well functioning immune systems. FluMist™ is currently available in the USA and is reported to show significant subtype specific immunity i.e. against antigenically drifted viruses within a subtype [8]. In this sense it mimics the immunity induced by natural infection better than parenteral vaccination with non-replicating virus. Three factors may be considered to be important in this context. Firstly, the generation of local immunity in the mucosa of the respiratory tract involving IgA and effector T cells is better achieved through nasal immunisation with a live attenuated virus such as FluMist™ than intramuscular injection with inactivated virus. Secondly it is not yet possible to induce effective immunisation through the intranasal route with recombinant proteins or non-replicating viruses without the use of strong adjuvants that are not licensed for this route of administration. Lastly, and from a safety point of view, nasal delivery carries a risk of viruses or vaccine components accessing the brain through the anatomical proximity of the olfactory epithelium and lobe, and of the vaccine inducing hypersensitivity reactions in the respiratory tract. The available information on the nature of protection against the different forms of the influenza virus resulting from natural infection and different forms of vaccination is summarized in Table 4.

A vaccine that is able to generate broadly neutralising antibodies against many different strains and subtypes is being sought by many researchers. A priming immunisation with a plasmid H1N1 HA – based vaccine boosted with seasonal H1N1 influenza vaccine or recombinant HA protein

Table 4. Cross-Protection with Current Vaccines and Natural Infection

Target for Immunity	Natural Infection	Parenteral Vaccine	FluMist™
Immunising or infecting strain	Very good	Very good	Very good
Different strains of a subtype	Good	Poor	Good
Different subtypes	Poor	Nil	Not Known
Different types e.g. A, B or C	Nil	Nil	Nil

was recently shown to elicit antibodies that were able to neutralize a range of HIN1 strains from 1934 to 2007 [9]. These antibodies reacted with a relatively conserved region in the stem of the haemagglutinin molecule, which is also known to be a target for broadly cross-reacting monoclonal antibodies to the haemagglutinin [2]. Because the peptide epitopes recognised by T_C in viral proteins present in the interior of the virion are less prone to antigenic drift caused by antibody selection, such proteins are also being investigated for the development of vaccines effective against diverse strains and subtypes.

Another feature of the antibody response to influenza A viruses is its ability to persist for long periods of time. Survivors of the 1918 H1N1 pandemic have been shown to possess antibodies that neutralized this strain and circulating memory B cells that were capable of becoming activated to produce specific antibodies 90 years later [10]. The antibodies produced were class switched and had undergone multiple somatic mutations to generate high binding affinity for the antigen [10]. Long lasting antibody responses are also characteristic of vaccines such as the Yellow Fever vaccine. Antigen-independent polyclonal activation of memory B cells and long-lived plasma cells may be responsible for the persistent antibody response seen in these elderly persons.

5. Immunopathology of the 2009 A (H1N1) Influenza Virus

Data from the Centre for Disease Control in the USA [11] indicates that approximately 61 million cases of infection were recorded in the USA between April 2009 when the first case was diagnosed and April 2010. There were also an estimated 274,000 hospitalizations and 12479 deaths during this period. The 2009 A(H1N1) influenza tended to affect more young people under the age of 30 than the very young or elderly which contrasts with the normal effects of seasonal influenza [12]. In the near term the virus is expected to establish itself as a seasonal influenza virus. Clinical data indicates that the 2009 A(H1N1) influenza caused somewhat more severe symptoms and lung pathology than the seasonal influenza viruses that had been circulating in the recent past [12, 13]. An unusual feature of 2009 A(H1N1) influenza infection is a greater incidence of severe viral pneumonitis, leading to acute respiratory distress syndrome. Its greater pathology compared to normal seasonal influenza strains is therefore partly attributable to infection and replication in lower respiratory tract. In this sense it resembled the 1918 H1N1 influenza strain. Experimental studies in macaques showed that the 1918 H1N1 influenza virus caused greater lung pathology compared to a conventional influenza virus control A/Kawasaki/173/01 (K173; H1N1) [14]. Studies on microarray-based analysis of gene expression in bronchial tissue showed that the 1918 H1N1 influenza virus induced an atypical innate immune response involving a reduced early Type 1 IFN response, compared to the conventional virus. It is speculated that this may be responsible to the greater disease severity associated with the 1918 H1N1 influenza virus [14]. It appears that the 2009 A(H1N1) influenza virus may also elicit an aberrant early innate immune response that results in less protection and greater disease severity [12-16].

There is evidence to suggest that differential tropism of influenza viruses in the respiratory tract may also play a role in disease severity. The upper respiratory tract epithelium of humans have mainly α 2-6 linked terminal sialic acid residues present at the ends of long glycan chains while birds have terminal α 2-3 sialic acid and α 2-6 sialic acid on shorter glycan chains that have a particular topology favouring the binding of avian influenza viral HA [17]. However human lower respiratory tract type II pneumocytes of humans contain α 2-3 as well as α 2-6 sialic acid-linked glycans. Although the 2009 A(H1N1) influenza HA preferentially binds α 2-6 sialic-linked glycans and to the trachea, it also binds to alveolar tissue [16], a property that may contribute to more prominent viral infection of the lower respiratory tract, systemic spread and consequent greater pathogenicity.

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Glucose-6-phosphate dehydrogenase deficiency among newborn in Brunei Darussalam

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Abstract

Glucose-6-phosphate dehydrogenase deficiency is the most common enzyme disorder in the world affecting up to 400 million people. This X-linked defect predisposes affected persons to haemolyis caused by oxidative stress. Resistance to malaria is linked with G6PD deficiency. Its incidence in Brunei Darussalam was estimated with data obtained from routine screening of newborn children during the period 2007 to 2009 at the RIPAS hospital using a semi-quantitative fluorescent spot test.

Prevalence of glucose-6-phosphate dehydrogenase deficiency among infants in Brunei Darussalam over the three years was 3%. The prevalence among male infants (n = 5245) was 5% (95% CI: 4%, 5%) with 253 detected cases. The prevalence among females (n = 5338) was 1% (95% CI: 1%, 2%) with 69 detected cases. There was no association between Chinese or Malay ethnicity and the deficiency of glucose-6-phosphate dehydrogenase (P=0.775). These data are comparable with results from Malaysia which has a population with a similar ethnic composition. Comparison with data from Malaysia also suggests that a photometric assay-based quantitation of enzyme activity detects milder forms of G6PD deficiency that are missed by the fluorescent spot test.

Key Words: *Brunei, glucose-6-phosphate dehydrogenase deficiency, newborn*

Introduction

Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is the most common inherited human enzyme deficiency in the world with as many as 400 million people carrying the defective gene. The defect is most prevalent in Africa (affecting up to 20% of the population), but is common also in the Mediterranean countries (4% - 30%) and Southeast Asia (0.5- 26%) [1-4]. It is an inherited disorder in which the G6PD gene on the X-chromosome is defective. G6PD is the first enzyme in pentose phosphate pathway that provides energy to the cells in the body, including erythrocytes, as well as maintaining the level of reduced nicotine adenine nucleotide phosphate (NADPH). NADPH detoxifies hydrogen peroxide through the glutathione pathway, thus neutralizing the oxidative stress to the cell. In patients with G6PD deficiency, this mechanism is impaired, making the cells more susceptible to oxidative damage. Red blood cells are prone to damage because of continuous change between deoxygenated and oxygenated states, which generates small amounts of superoxide anions. Exposure of blood to exogenous oxidizing agents, e.g. drugs such as primaquine and foods such as fava beans, and infections, can lead to haemolytic anaemia. Other clinical manifestations associated with G6PD deficiency are neonatal jaundice and sometimes chronic non-spherocytic hemolytic anemia. Management of G6PD deficiency involves early detection through screening of newborn and the avoidance of oxidative stress in deficient persons [1-4].

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The World Health Organization has classified the different G6PD variants according to the magnitude of the enzyme deficiency and the severity of hemolysis. Classes IV and V are of no clinical significance. Class I patients are rare, have severe enzyme deficiency (less than 10% of normal enzyme activity) and have chronic hemolytic anemia. Class II patients have severe enzyme deficiency but there is usually only intermittent hemolysis. Class III patients have moderate enzyme deficiency (10 to 60% of normal) with intermittent hemolysis that is usually associated with infection or drugs. Class IV patients have no enzyme deficiency or hemolysis and Class V patients have increased enzyme activity [4].

X-Chromosome inactivation and G6PD deficiency

G6PD deficiency is an X-linked disorder involving a gene located in the terminal region of the long arm of the X chromosome (Xq28). Red cells in heterozygous females with G6PD deficiency were found to be mosaic within two populations: one with normal G6PD levels and the other with deficient G6PD levels. Random inactivation of one of the X chromosomes in haemopoietic stem cells due to Lyonisation is the cause of the mosaicism. Carrier females with only 50% G6PD activity are also prone to haemolysis [1-4].

G6PD deficiency and protection against malaria

Despite the negative consequences of G6PD deficiency, defective genes have persisted in many parts of the world [1-4]. Different mutations giving rise to inactive or less active enzyme are found in different regions that are affected or have been affected in the recent past by malaria, suggesting strong evolutionary selection [1, 3]. The role of G6PD deficiency in protecting against *Plasmodium falciparum* was elegantly demonstrated by the preferential growth of the parasite in normal red cells, and not G6PD deficient red cells, in heterozygous female blood [5]. However field studies in Africa suggest that protection against *P. falciparum* was significant only in hemizygous males and not heterozygous females [6].

Both *P. falciparum* and *Plasmodium vivax* are common parasites in Southeast Asia. Investigations on the relationship between the Mahidol mutation, a G6PD deficiency gene with an allele frequency of 12% in Thailand, and the *Plasmodium* parasite density in Thailand showed that it was significantly associated with reduced *P. vivax* but not *P. falciparum* density in blood [7]. It was hypothesized that *P. vivax* preferentially infects reticulocytes which have a high level of glutathione, an antioxidant tripeptide, to protect it from oxidative stress. On the other hand, *P falciparum* has no preference to reticulocytes, indicating that it is less sensitive to oxidative stress. Therefore, a person with reduced G6PD activity would be prone to oxidative stress and this would have a greater effect on *P. vivax* than *P. falciparum* [7]. It is also reported that G6PD deficient red cells parasitized by *P. falciparum* are more readily phagocytosed to provide a degree of protection against malaria [8]. The relative protection provided by G6PD deficiency against *P. falciparum* and *P. vivax,* and indeed the other species of human malaria parasites, is therefore not well established and is confounded by the fact that the prevalence of the two major parasite species has changed over time in different parts of the world.

G6PD deficiency assessment in Brunei

All newborn children are screened for G6PD deficiency to allow early detection and medical intervention. However there is little published data on the prevalence and incidence of G6PD deficiency in Brunei. Ethnic differences are possible because of differential exposure to malaria parasites among the distinct founding communities over the centuries and therefore a differential selection pressure. In addition, the traditional food preferences are not the same among the different communities in Brunei Darussalam. The screening method used in Brunei is a semi-quantitative fluorescence spot assay for G6PD activity based on the formation of NADPH after the addition of G6P and NADH to blood spotted on filter paper, followed by illumination with 340nm UV light. The fluorescence is determined visually with samples showing NADPH fluorescence termed normal and those not showing fluorescence termed G6PD deficient. In principle, this method, which was described initially by Beutler and Mitchel [9], can have a sensitivity of 100% and specificity of 99% in homozygous females and hemizygote males [10]. In heterozygote females however the sensitivity and specificity are reported to be 32% and 99% respectively [10].

The incidence of G6PD deficiency among newborn in Brunei Darussalam estimated with data in the period 2007 to 2009 is presented in this report.

Materials and Methods

This was a retrospective study on all babies born in RIPAS, among whom were babies confirmed to have G6PD deficiency through the routine neonatal screening tests for the period 2007 to 2009. The tests were carried out by the clinical chemistry laboratory at RIPAS. The data collected from birth records included the gender and ethnicity. Details of every newborn baby diagnosed with G6PD deficiency were recorded together with the total newborn population into a database. A list of code numbers, rather than names, was created to safeguard privacy. This study had the approval of the Ministry of Health Ethics Committee.

Statistical analysis

Data was entered and analyzed using the SPSS version 16.0 statistical software.

Laboratory test for G6PD deficiency

All samples were routinely tested by RIPAS hospital staff using a semi-quantitative fluorescent spot method for detection of deficient types using a kit made by Randox Laboratories (Antrim, UK). This method is recommended by the International Committee for Standardization in Hematology as a simple method of screening for G6PD deficiency and is stated by the manufacturer to have high sensitivity in high to moderate G6PD deficiency but low sensitivity in mild G6PD deficiency.

Results

Table 1. Percentage prevalence of G6PD deficiency amongst neonates in the period 2007-2009

Year	Variable		Prevalence among males (95% CI)	Prevalence among females (95% CI)	Total prevalence (95% CI)
2007	Ethnicity				
		Malay	5 (3, 6)	1 (0, 2)	3 (2, 4)
		Chinese	6 (0, 13)	2 (- 2, 5)	3 (0, 6)
		Others	9 (2, 16)	4 (0, 8)	5 (2, 8)
2008	Ethnicity				
		Malay	4 (3, 5)	1 (1, 2)	3 (2, 3)
		Chinese	1 (- 1, 3)	1 (- 1, 4)	2 (0, 4)
		Others	9 (4, 13)	2 (0, 4)	4 (2, 6)
2009	Ethnicity				
		Malay	6 (5, 7)	1 (1, 2)	3 (3, 4)
		Chinese	2 (0, 6)	3 (0, 7)	3 (1, 6)
		Others	3 (0, 5)	1 (0, 2)	1 (0, 3)

G6PD deficiency and other categorical variables

Categorical variables were analysized using Chi-square to determine any association with G6PD deficiency (Table 2)

Table 2. Factors associated with G6PD deficiency in the period 2007 to 2009

Variable	***n***	**G6PD deficiency *n* (%)**	**Normal *n* (%)**	**χ^2 statistic (*df*)**	***P* value**
Gender					
Male	5245	253 (4.8)	4992 (95.2)	111.8 (1)	<0.001
Female	5338	69 (1.3)	5269 (98.7)		
Ethnicity					
Malay	11178	332 (3.0)	10846 (97.0)		
Chinese	561	15 (2.7)	546 (97.3)	0.561 (2)	0.775
Others	870	29 (3.3)	841 (96.7)		

**Missing for gender = 2026; n = numbers ;*

The detected incidence among males was significantly higher than in females (P<0.001) as would be expected of an X-linked recessive gene. However, the incidence for Malay, Chinese and Others (others were Indians, Caucasians, and indigenous Bruneians) were not significant different (P = 0.775). Malays, Chinese and Others account for 73.8%, 14.8% and 1.4% of the population of Brunei respectively [11].

Discussion

Sensitivity of the screening test

The incidence of G6PD deficiency among newborns in RIPAS hospital may be taken as an approximate indication of its prevalence among the population of Brunei - Muara district, which is the major catchment area for births at RIPAS hospital. Brunei – Muara district is the most populous in Brunei, with approximately 66% of the total population of the country [11] and therefore the prevalence may also be taken to be approximately indicative of the whole country. The results show that the Randox test is detecting a proportion of females heterozygous for defective G6PD genes. The prevalence of G6PD deficiency reported here for Brunei is similar to the 3.3% detected in Malaysia using the same method [12]. However, the prevalence of G6PD deficient mutants in Myanmar (0-10.8% in different ethnic groups), Laos (7.2%) Indonesia (6%) was higher when direct DNA-based analysis for mutants was carried out [13].

A comparison was made in Malaysia between the efficiency between the Randox semi-quantitative fluorescent spot test and quantitative enzyme assay for detecting G6PD deficiency [12]. The semi-quantitative fluorescent spot test is based on the fluorescent appearance of reduced pyridine nucleotide (NADPH) when activated by UV light. Appearance of fluorescence indicates sample is normal whereas if fluorescent is not seen, the sample is said to be G6PD deficient. Enzyme assay is a quantitative test involves lysis of red cells followed by incubation of the lysates with substrate and the cofactor NADP, and subsequent photometric measurement of NADPH formation at 340 nm [12]. The prevalence of G6PD deficiency with the semi-quantitative fluorescent test and enzyme assay were found to be 3.3% and 7.2%, respectively [12]. Therefore the semi-quantitative Randox test failed to detect 3.9% of neonates with G6PD deficiency in that study. This may also be the case in Brunei Darussalam. Use of the more sensitive photometric enzyme assay test will help detect the milder form of G6PD deficiency, be helpful in management of potential neonatal jaundice, and identifying all of the deficient individuals for studies on types and prevalence of mutations at the DNA level. Furthermore when a G6PD deficiency based on enzyme assay was used to test protection against clinically uncomplicated malaria caused by *P. falciparum,* African females were found to be significantly protected [14] in contrast to genetic typing of females carrying mutant G6PD [6]. This further highlights the importance of using the appropriate assay for G6PD deficiency in epidemiological studies.

G6PD variants in Southeast Asia

There are numerous types of G6PD variants in Southeast Asia that are related to the severity of hyperbilirubinemia as well as the onset of jaundice. G6PD mutations and their G6PD activity were identified through genetic analysis and enzyme assays, respectively. The three most common alleles G6PD Viangchan, Mediterranean and Mahidol were reported to cause at least 80% of G6PD deficiency in Malaysian Malays [15] and G6PD Mahidol and Viangchan are present elsewhere in Southeast Asia [13]. Investigation of these and other alleles in Brunei and elsewhere in Borneo island may yield useful information pertaining, for example, to population migrations, habitat in relation to present and previous malaria exposure, and preferences for traditional medicines and foods.

Competing Interests

The authors declare that they have no competing interests.

Author Contribution

RR conceived the project and wrote the manuscript. PUT supervised clinical aspects of the project and LKCA collected and analysed data.

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Epidural use during childbirth at the RIPAS hospital in Brunei

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Abstract

Epidural analgesia is a commonly employed technique of providing pain relief during labour. Despite the high delivery rate in RIPAS Hospital the use of epidural analgesia is limited compared to Western nations where it ranges from 25 – 50%. We analysed the epidural figures of RIPAS hospital retrospectively for the year 2008 using descriptive statistics to look into the indications, complications, outcome and awareness to identify methods to increase the use of epidural analgesia. The number of patients who opted for the use of epidural was only 1.4% of the patients who were admitted in labour ward, RIPAS Hospital in the year 2008. Majority of the users were primigravidae (51%) and the main indication was pain. Our study showed an increase in both caesarean (26.5% vs. 15.2%) and instrumental delivery (7.8% vs. 1.5%) rates compared to patients who did not undergo epidural. It is proposed that more information on epidural use be given to women antenatal checks.

Keywords: *Childbirth, Epidural analgesia, Brunei Darussalam*

1. Introduction

Epidural analgesia is a commonly employed technique of providing pain relief during labor. The number of parturients given intrapartum epidural analgesia is reported to be around 25% in UK to >50% in many institutions in the United States [1, 2]. The procedure has few contraindications, the primary ones being patient refusal, maternal haemorrhage and coagulopathy. Induction of epidural analgesia in early labor remains controversial. However, many physicians induce analgesia as soon as the diagnosis of active labor has been established and the patient has requested pain relief. Retrospective studies have demonstrated an association between epidural analgesia and increases in the ideal duration of labor, instrumental vaginal delivery and caesarean section for labor [3-5]. However, several recent prospective studies have concluded that epidural analgesia does not adversely affect the progress of labor or increase the rate of caesarean section [6.7]. These remain controversial issues among practicing physicians. The most common complications occurring with epidural analgesia are maternal hypotension and postdural puncture headache [8,9]. Epidural analgesia became available round the clock in RIPAS Hospital labour room in 2007. But despite the high delivery rate in RIPAS Hospital the use of epidural analgesia is limited.

This study analysed epidural usage statistics at RIPAS hospital to look into the common indications, complications, outcome and awareness of epidural availability. This was done to identify methods to increase the use of epidural analgesia in RIPAS Hospital, Brunei

2. Materials and Methods

In this retrospective study, labour room records were analysed for the year 2008 to identify the women who had undergone epidural analgesia during labour. Then information was collected from epidural register and case notes. Awareness of the use of epidural analgesia among the women was obtained from records maintained by anaesthetists. Data was collected regarding the parity, mode of delivery, duration of labour, indications for epidural, awareness, satisfaction and complications. Time between decision and insertion of epidural was also analysed.

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3. Results

The total number of cases where epidural was used was 64 out of 5051 deliveries that occurred in RIPAS Hospital in the year 2008. When the number of elective caesareans (n=390) were excluded this was a very low 1.4%. Only 8 patients had antenatal knowledge of the epidural analgesia out of which 4 were medical staff. 51% of the epidural users were primigravidae and 41% were gravida 2-4. Only 8% were >gravida 5. All the epidurals were inserted in the 1st stage of labour. Pain accounted for 70% (n=45) of the indications while only 30% (n-19) were due to maternal requests. Among the 19 maternal requests, 11 were primigravidae, 7 were gravida 2-4 and only 1 was >gravida 5. Among the 45 women who requested epidural due to pain, 22 were primigravidae, 19 were gravid 2-4 and only 3 were >gravida 5. In 69% (n=44) the time taken between decision and insertion of epidural was between 30 minutes to 1 hour. Twelve percent (n=8) took more than 1 hour. Forty two patients delivered normally, while 5 had instrumental deliveries and 17 had to undergo caesareans. All the instrumental deliveries and 12 of the caesareans were primigravidae. Five caesareans had to be done in the gravida 2-4 group. All the >gravid 5 delivered spontaneously. More than 95% of the patients were satisfied with the procedure with only 2 reporting unilateral blocks and 1 patient had nausea. One procedure had to be abandoned due to dural tap. The mean duration of labour in primigravidae who were given epidural was 5 hrs 38 minutes (range from 45 minutes to 10 hours 43 minutes) which was not increased compared to textbook statistics. But the incidence of emergency caesareans (26.5%) and instrumental deliveries (7.8%) was raised compared to the patients who did not have epidural analgesia (15.2% and 1.3% respectively).

4. Discussion

Despite the high number of deliveries in RIPAS Hospital, Brunei, the 1.4% use of epidural analgesia contrasts with countries like UK and USA where it ranges from 25-50%. The reason could be poor awareness among the public and lack of antenatal counselling on epidural use in Brunei. Fifty one per cent of the epidural users in Brunei were primigravidae and 41% were gravida 2-4. Only 8% were >gravida 5. The majority of indications in our study were due to pain (70%). The complications were negligible in our series. The duration of labour was not increased in primigravidae when compared to the ideal Friedman's curve. The increase in caesarean rate could be attributed to the small number of cases who had undergone epidural analgesia. The increase in instrumental deliveries is expected according to international literature. The limitation of our study has been the small number of epidural cases that did not permit detailed statistical analysis.

To improve the situation in Brunei, stress should be laid on imparting knowledge of epidural use to women during antenatal checks e.g. through the use of brochures. Currently, a questionnaire has been developed and will be used to assess patient attitudes to epidural analgesia and also to determine cultural and socio-economic influences on its use.

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Isolated non-chylous congenital pleural effusion in a neonate – case report and review of literature on current prenatal and postnatal management

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Abstract

Respiratory distress is a leading cause of mortality and long term morbidity in neonates. Hyaline membrane disease, meconium aspiration, pneumonia, diaphragmatic hernia and cystic adenamatoid malformations are the commonest cause of respiratory distress in pre-term and term babies respectively. Pleural effusions are a rare cause of respiratory distress in new born babies. Most of these effusions are chylous. Chylous effusions are mostly seen in babies with Down's syndrome. Non-chylous effusions are rare and early recognition is crucial because it has an excellent prognosis on early diagnosis and treatment. A case of congenital non-chylous pleural effusion in a baby born in Brunei Darussalam is described and its clinical significance discussed.

Key Words: *chylothorax, pleural effusion, thoracentesis*

Introduction

Congenital pleural effusions are extremely rare in neonates. Most of the reported cases of congenital pleural effusions are chylous [1]. Antenatal diagnosis is important as early neonatal diagnosis and intervention could reduce mortality and morbidity. Adequate respiratory and hemodynamic support and drainage of the effusion gives excellent results in non-chylous effusions. Chylothorax is treated with thoracentesis and feeds with medium chain triglyceride containing formula. We report our experience with a case of congenital non-chylous pleural effusion in a baby that was diagnosed in an antenatal ultrasound scan.

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Case Report

A term female baby weighing 3100g was born to a gravida 2 mother by caesarean section. Mother had regular antenatal care and her serology was negative for HIV, Hepatitis B & C and syphilis and was immune to rubella. Her high vaginal swab culture was sterile. Her fetal ultrasound scans at 18 and 33 weeks were normal. A repeat fetal scan at 36 weeks showed a right congenital pleural effusion [Fig.1].

At birth, the baby cried spontaneously but became dusky one minute after birth. She required bag and mask ventilation followed by endotracheal intubation. The baby was transferred to the neonatal intensive care unit and mechanical ventilation was started. Umbilical catheters were inserted and the baby was stabilized with moderate conventional ventilator settings. A chest X-ray confirmed the prenatal ultrasound finding of a moderately large right sided pleural effusion [Fig.2]. An ultrasonogram of the chest showed the right lung surrounded by fluid with a shift of the mediastinum to the left. Two dimensional echocardiography, renal and head scans were normal. At 7 hours of life, a chest drain was inserted and 80 ml of fluid was drained [Fig.3].

Analysis of the pleural fluid showed an exudative effusion with a protein level of 35g/L, LDH 609 IU/L, triglycerides 0.31mmol/L, WBC 4640/mm3 with 72 % neutrophils and 28% lymphocytes. A repeat pleural fluid analysis on day 6 revealed protein 27g/L, LDH 2554 IU/L, triglycerides 0.59mmol/L, WBC 640/mm3 with 80% neutrophils and 20% lymphocytes. Culture and gram staining of the pleural fluid were negative for pathogens in both samples. Blood counts, C reactive protein, renal panel, liver function tests and coagulation profile were within normal limits. Blood and surface swab cultures were negative. Antibiotics were stopped on day 6. Karyotyping confirmed a 46XX normal chromosomal pattern. Chest X-ray on day 5 showed total clearance of pleural fluid.

By day 6, there was minimal amount of pleural fluid drainage and the chest drain was subsequently removed on day 6 with a total of 140ml of fluid drained. The baby was weaned off the ventilator and extubated at 52 hours of life. She was breastfed on day 4 and was on full feeds on discharge from the unit. A repeat chest X-ray on day 10 [Fig. 4] showed normal lung fields. She was discharged home. On review at 1 and 6 month of age, the baby remained well and asymptomatic with adequate weight gain.

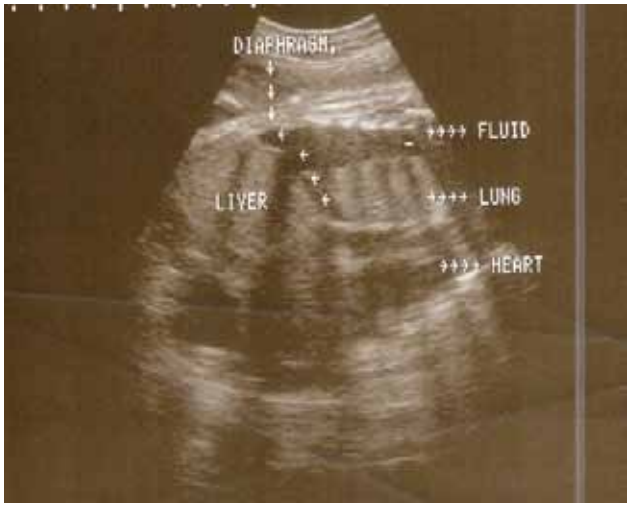

Figure 1. Antenatal scan showing pleural effusion

Figure 2. Xray at 2 hours of life with right sided pleural effusion

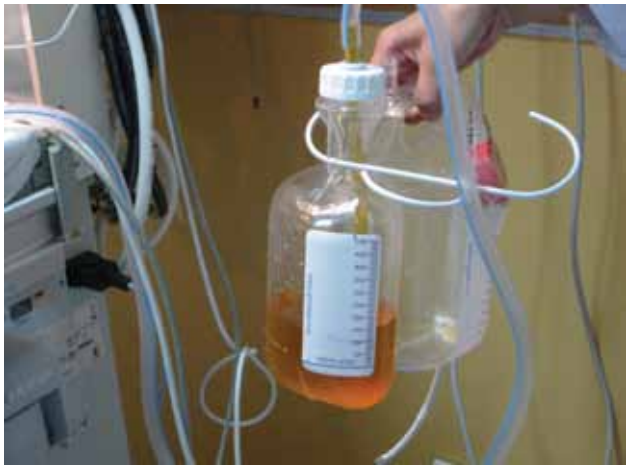

Figure 3. Drained pleural fluid from the right chest

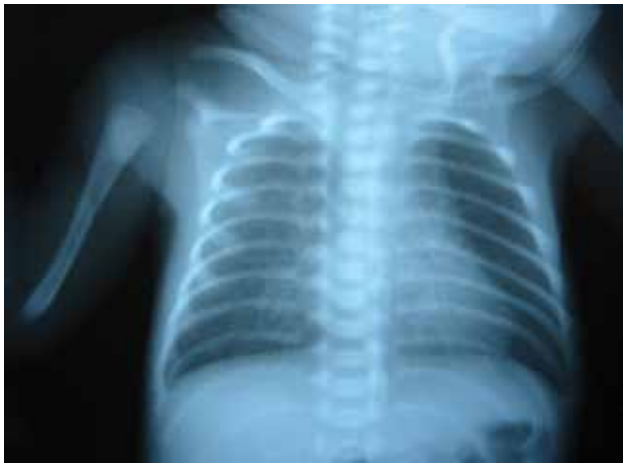

Figure 4. Xray chest on day 10 showing normal lung fields

Discussion

Congenital pleural effusions are rare in neonates and have been reported in 1:12000 to 15000 pregnancies [5]. Causes include hydrops fetalis, intra-uterine pneumonia, cardiac abnormalities, intra-thoracic or cardiac surgery, leakage from long lines and trauma [1, 2, 6].

Most of the congenital pleural effusion fluids are chylous in nature, resulting from a malformation or tear in the fetal thoracic duct and 60% of cases has been found in the right hemithorax [1]. Chyle is noninflammatory, lymphocyte predominant fluid having low LDH and reported as protein discordant exudates [10]. Chylous effusions may be initially serous and turns milky after feeding or initiation of total parenteral alimentation. Chromosomal anomalies like Down's syndrome and Turner's syndrome have been reported in association with neonatal chylothorax [1, 4].

Non-chylous pleural effusions however are rare and have been reported in babies with underlying conditions like primary lymphangiectasia, congenital cystic adenamatoid malformation, bronchopulmonary dysplasia, diaphragmatic hernia and pulmonary vein atresia and rarely Down's syndrome [9]. Non-chylous effusion seen in our case did not isolate any pathogens from blood or pleural fluid. Protein and cell count were high. There was no underlying lung pathology.

Fetal ultrasound scans has increased the number of antenatally detected cases with congenital pleural effusions as in this reported case. Chest radiograph in babies with significant pleural effusion usually demonstrates obliteration of the costophrenic angle and a homogenous haziness in the affected hemithorax with contralateral mediastinal shift [6]. Diaphragmatic hernia with fluid filled loops of gut, congenital cystic adenamatoid malformation or pulmonary sequestration and total atelectasis should be considered in the differential diagnosis. Ultrasonograpy of the chest is a useful non invasive tool for diagnosis of pleural effusion and underlying lung conditions [2, 6, 8].

Thoracentesis and fluid analysis are necessary for the diagnosis. Chylous effusion is milky-white after milk feeds or haemoserous in colour with an absolute cell count of greater than 1000/mm3, lymphocyte fraction greater than 80% and triglyceride level >1.1mmol/l [3]. Non chylous effusion could be a transudate or exudate. Transudates have total protein less than 30g/L and WBC fewer than 2000/mm3 with a predominance of mononuclear cells where as exudates have high protein levels and predominance of polymorphonuclear cells [9]. Exudative fluid may show the pathogen in gram stain or culture. The pleural fluid in our case was an exudate but sterile.

Fetal pleural effusions, if diagnosed antenatally, need to be followed up closely. These effusions may undergo spontaneous resolution or increase over time resulting in tension hydrothorax [4]. Tension hydrothorax will require intra-uterine thoraco-amniotic shunting in order to prevent pulmonary hypoplasia or even intra-uterine death. Neonates born with moderate to large effusions require early and active intervention. Intubation and mechanical ventilation will help to resolve early respiratory insufficiency. Thoracocentesis for decompression of the pleural effusion should be performed. Fluid reaccumulation following thoracocentesis should be treated with a chest drain. Antibiotics should be given to all neonates presenting with effusions at birth and continued until an infectious aetiology has been excluded [3].

For congenital chylothoraces, majority of the published literatures support a treatment regimen beginning with a period of parenteral nutrition followed by medium chain triglyceride containing formula. Somatostatin or its analogue octreotide has been reported with success in chylothorax not responding to chest tube drainage and bowel rest [7].

In conclusion, early recognition of neonatal pleural effusion is crucial because of the excellent prognosis following timely diagnosis and treatment.

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Paraganglioma of cauda equina - case report and literature review

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Abstract

Paragangliomas are tumors arising from neuroectodermally derived extra-adrenal paraganglion system which has a wide distribution throughout the body. The majority of paragangliomas are located in the head and neck region. Their occurrence in the spinal canal is infrequent. We report the first case of paraganglioma of cauda equina from Brunei Darussalam. The histological features and immunohistochemical findings are discussed.

Keywords: *cauda equina, immunohistochemistry, paraganglioma*

Introduction

Paragangliomas are uncommon tumours arising from neuroectodermally derived extra-adrenal paraganglion system which has wide distribution throughout the body. The majority of paragangliomas are located in the head and neck region and arise from paraganglia intimately associated with parasympathetic nervous system. Examples of such tumours are carotid body tumour, glomus jugulare tumour, vagal body tumour etc. Those extra-adrenal paragangliomas arising from aorticosympathetic paraganglia associated with sympathetic nervous system occur predominantly in the para-aortic region of retroperitoneum including anatomic sites of organs of Zuckerkandl, posterior thorax and neck [1, 2]. They may rarely occur in urinary bladder, prostate and gall bladder [3-5]. Primary paragangliomas occur infrequently in the spinal canal [6-12]. We report a case of paraganglioma of cauda equina.

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Clinical presentation

A 26 year-old policeman presented with a four-month history of pain in the lower lumbar region and right sciatica. He also complained of weakness and numbness of both legs which predominantly affected the left side. He also experienced gait difficulties. He denied any past history of spinal trauma. His bladder function was intact. No vasomotor symptoms including episodic hypertension were present. The blood pressure was 120/80 and pulse rate was 68/min throughout his stay in hospital. Neurologically, he was having 4/5 power in the right hip, knee and ankle. Deep tendon reflexes were absent in both legs. Straight leg raising test was negative. He also had L3, 4, 5 and S1 dermatomal hypoesthesia. Plain radiograph of lumbar spine showed no obvious abnormality. However, magnetic resonance imaging (MRI) of lumbar spine revealed a well-defined intradural extramedullary enhancing lesion in the D12-L1 region pushing the conus medullaris upwards and compressing the cauda equina (Fig. 1). The MRI diagnosis was possibly a schwannoma or ependymoma. D12 and L1 laminectomy was subsequently performed. The lesion was seen on opening the dura mata and it was found to be attached to one of the nerve roots with a vascular pedicle. The vascular pedicle with nerve root attachment was cut, cauterized and tumour was resected completely. The operation was uneventful without any complications. Postoperatively, the patient had a complete neurological recovery. No signs and symptoms of recurrence were noted at 20 months follow-up.

Pathological features

Grossly, the tumour was small, ovoid, dark brown and soft to relatively firm in consistency. It measured 2x1.5x1cm. Cut surface showed yellowish to dark tan appearance (Fig. 2). Routine histological examination showed a small tumour enclosed by fibrous capsule. It exhibited typical nesting (Zellballen pattern) of neoplastic chief cells surrounded by delicate fibrous septa richly endowed with congested dilated sinusoidal capillary network. The chief cells were generally uniform, medium-sized and polygonal in appearance. The nuclei were round and vesicular with finely dispersed chromatin. Mild degree of nuclear atypia was noted. The cytoplasm was pale pink granular to homogenous eosinophilic in nature. Only occasional scattered mitoses were present. The flattened to spindle sustentacular cells were seen at the periphery of nests of tumour cells. The chief cells were also found to be arranged into anastomosing strands and cords in areas. Microcystic change and presence of some siderophages were noted in a few areas. There was no evidence of vascular and capsular invasion (Fig 3). Immunohistochemical studies showed diffuse positive immunoperoxidase reactions of chief cells for neurone specific enolase (NSE), chromogranin A, synaptophysin and vimentin . The sustentacular cells were highlighted by positive reactions to S100 protein and glial fibrillary acidic protein (GFAP). The chief cells were found to be negative for GFAP and neurofilament protein (NF) (Figs. 4-6). These typical histomorphologic features and positive immunoreactions for neuroendocrine markers established the diagnosis of this tumour as paraganglioma.

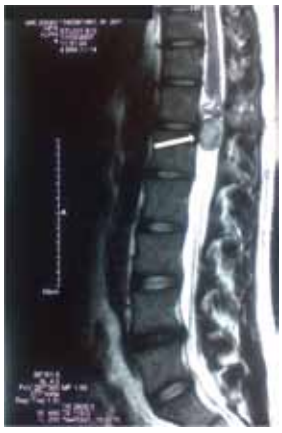

Figure 1. T2 weighted MRI of thoracolumbar spine showing an intradural lesion (white arrow) at T12/L1 level compressing and displacing the conus medullaris superiorly.

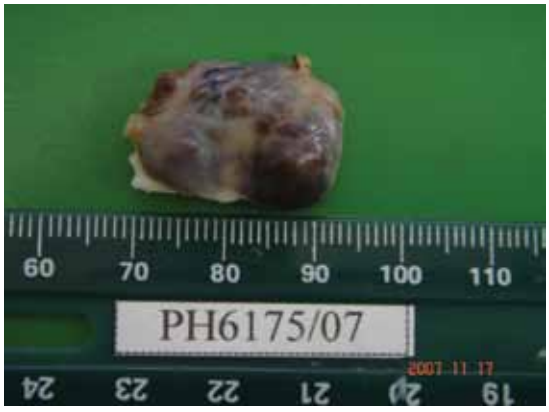

Figure 2. Gross appearance of the resected tumour. It was small, ovoid, dark brown and measured 2x1.5x1cm.

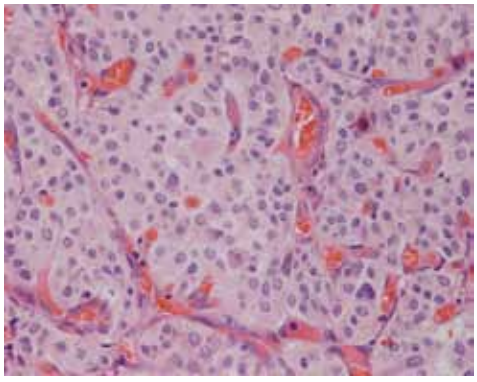

Figure 3. Histology of the tumour showing typical Zellballen pattern of chief cells surrounded by richly vascular stroma (H & E, original magnification x200).

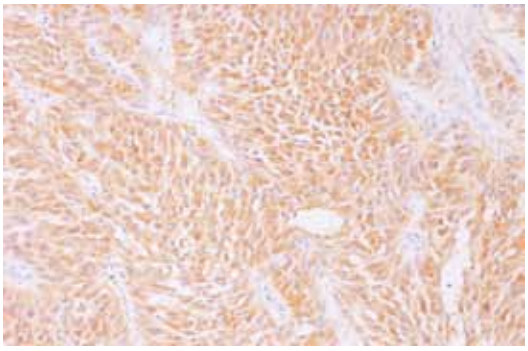

Figure 4. Immunoperoxidase stain for neurone specific enolase demonstrates diffuse dark brown cytoplasmic positivity of chief cells (original magnification x200).

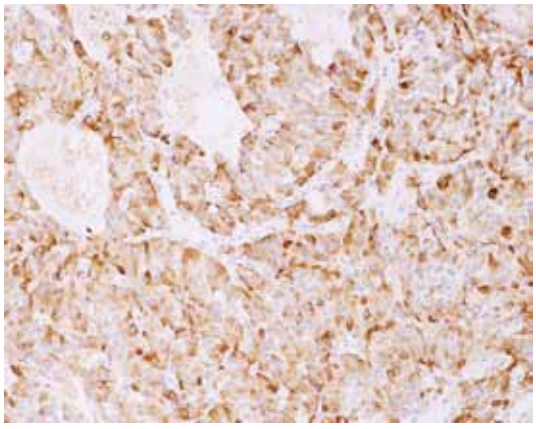

Figure 5. Immunoperoxidase stain for chromogranin A displays granular dark brown cytoplasmic positivity of chief cells (original magnification x200).

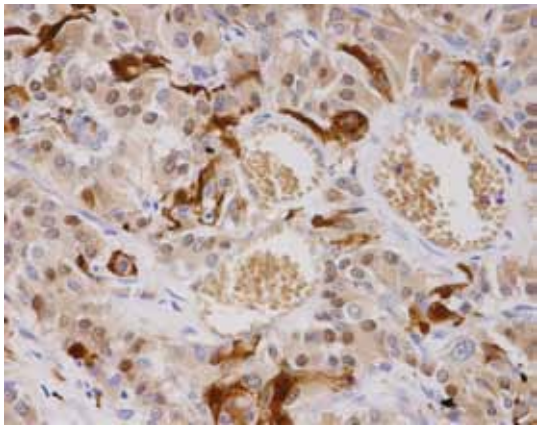

Figure 6. Immunoperoxidase stain for S100 protein highlights the spindle sustentacular cells (original magnification x400).

Discussion

Paragangliomas occur infrequently in the spinal canal. The majority are located in lumbar region, cauda equina and filum terminale [6-12]. The lumbar and cauda equina paragangliomas are presumed to arise from neural stem cells present in the ependymal layer of the spinal canal or from the residual peripheral neuroblasts residing in cauda equina [13-15].

The recognition of spinal paraganglioma as a distinct clinical and pathologic entity by its histological, histochemical and ultrastructural characteristics was reported first by Lerman et al in 1972 as a ganglioneuroma-paraganglioma of cauda equina in a 29 year-old black man [16]. In fact, a case of a "secretory ependymoma" of filum terminale reported by Miller and Torack in 1970 was in retrospect proven to be the first case of spinal paraganglioma [17]. Since then only limited numbers of single case reports and small case series were reported in literatures [6-12]. A total of 184 cases of lumbar paraganglioma had been reported until 2008 [18].

Most of these tumours clinically presented with low back pain with or without sciatica and other clinical signs and symptoms of spinal cord compression [7, 8, 10-12]. Uncommon signs and symptoms of increased intracranial pressure may also occur [19]. The patient's age ranges from 9 to 74 years (mean 45.9 ±13.1 years). Males are affected more than females[10]. However, no gender difference was noted in the study of 30 cases reported by Moran, Rush and Mena [8]. The clinical signs and symptoms are non-specific and usually these will initiate radiological investigations including magnetic resonance imaging (MRI). MRI findings are also found to be generally non-specific but according to Abe et al, the presence of serpiginous vasculature and a vascular pedicle is diagnostic if visible on MRI [20]. Correlation between MRI findings and pathologic features of cauda equina paragangliomas was also described by Yang et al in their study of clinicopathoradiologic finding in four cases [21].

The definitive diagnosis of paraganglioma depends on the recognition of typical histomorphologic appearance on routine haematoxylin and eosin (H and E) histology and positive immunohistochemical reactions of tumour cells for neuroendocrine markers. Clinicopathological, histomorphological and immunohistochemical studies of spinal paragangliomas were reported by Sonneland et al in 1986 and by Moran, Rush and Mena in 1997 [7, 8]. A comprehensive review of histology, ultrastructure, immunohistology and molecular biology of extra-adrenal paragangliomas was reported by Kliewer and Cochran in 1989 [22].

From these studies as well as from other reported cases of extra-adrenal paragangliomas, it is found out that the basic histomorphologic features of all paragangliomas are similar whether they are situated in spinal canal or other sites.

The typical features are dual-cell population and arrangement of chief cells into compact nests (Zellballen) surrounded by delicate fibrous septa richly endowed with sinusoidal capillary network. The nests of chief cells are wrapped by spindle sustantacular cells in the peripheral region. Immunohistochemical studies of paragangliomas displayed positive immunoreactivity of chief cells for neuroendocrine markers such as NSE, chromogranin A and synaptophysin. Sustentacular cells are immunoreactive for S100 protein and GFAP.

The spinal paragangliomas are usually non-functional although they are of neuroendocrine origin. Only three cases of functionally active cauda equina paragangliomas were found in the literature reviewed by Gelabert-Gonzalez in 2005 [10]. Most of spinal paragangliomas are slow growing, benign in nature and less than 1% may be locally aggressive [8].

The differential diagnosis of paragangliomas of cauda equina includes myxopapillary ependymoma, neural tumour, meningioma and metastatic carcinoma. Among them, the lesion most commonly confused with paraganglioma on routine H & E histology is myxopapillary epdendymoma. Misinterpretation of paragangiloma as ependymoma is contributed by the fact that paraganglioma may exhibit intimate cell-capillary relationship as well as formation of perivascular pseudo-rosettes and papillae. It is possible differentiate paraganglioma from ependymoma by immunohistochemical studies. The tumour cells of myxopapillary ependymomas are negative for neuroendocrine markers but are positive for GFAP and S100 protein. The differentiation of paraganglioma from ependymoma is important as paraganglioma is slow growing, benign in biologic behavior and complete excision of the tumour will be curative in contrast to ependymoma.

Our patient is a young man who presented with lower lumbar pain associated with right sciatica, bilateral numbness and weakness of the legs of four months duration. MRI of lumbar spine detected a well-defined enhancing intradural extramedullary tumour in D12-L1 region. Histomorphology and immunohistochemical studies showed typical features of paraganglioma. Following the total resection of the tumour, he recovered completely from neurological signs and symptoms and is back at work as a policeman. He is also found to be free from recurrent neurological signs and symptoms at 20-month follow-up.

Conclusion

Paragangliomas occur infrequently in spinal canal in contrast to their more common occurrence in the head and neck region. Spinal paraganglioma should be considered as one of the differential diagnoses of spinal intradural tumours such as ependymoma, neural tumour and metastatic carcinoma. We report this case to be aware of the occurrence of paraganglioma in spinal canal which can be cured surgically if completely excised.

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Interprofessional education: the growing edge of teaching and learning in medicine

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Abstract

The aim of interprofessional education is to improve interprofessional practice. The Universities of Southampton and Portsmouth have been offering a combined programme of interprofessional education to all healthcare students since 1999. This article describes some of the main features of this course, and considers our experiences in its implementation in the context of the wider literature on the subject. The potential roles of educational theory, research, student leadership and assessment are explored.

Key Words: *Interprofessional healthcare education, University of Southampton*

Interprofessional education is a concept that has been around for many years. In 1988, a study group of the World Health Organization (WHO) met in Geneva; their report brought together the experiences of many individuals already working in the field [1]. Since then, it has become established as an important component of many curricula in medicine and the health sciences [2, 3], and this article will explore some of the issues involved in implementing interprofessional education. It will draw on our experiences at Southampton.

What is it, and why?

Interprofessional education involves students of different disciplines learning collaboratively. It has been defined thus [2]:

"Interprofessional education occurs when two or more professions learn with, from and about each other to improve collaboration and the quality of care."

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As such, interprofessional education needs to be distinguished from the situation where students of different disciplines learn alongside each other (e.g. by attending the same lecture), but not from each other [4]. This may be called "learning in common" to differentiate it from "interprofessional education" [5]. Learning in common has a valid place in the curriculum by delivering learning outcomes that are shared by different disciplines, but it is not interprofessional education because the students are not learning from one another.

The rationale underpinning interprofessional education is the hypothesis that it will lead to improved interprofessional practice, which will lead to improved health outcomes [6]. This principle was summed up as long ago as 1988 by the WHO [1]:

"Multiprofessional education is not an end in itself but a means of ensuring that different types of health personnel can work together to meet the health needs of the people."

In the United Kingdom, the idea that health care workers of different disciplines need to work together better was given impetus by high profile examples of perceived failures in collaboration between professional groups. In particular, the Kennedy Report into paediatric heart surgery in Bristol [7] and the Laming Report into the care of a child who was abused and murdered [8] both identified

dysfunctional interprofessional relationships as a significant problem: individuals who possessed important parts of the jigsaw, so to speak, did not put them together so the overall picture could become clear. Proponents of interprofessional education believe it has a role in preventing such tragedies.

Interprofessional education can occur at any stage in the career of a healthcare worker, from the earliest days of the pre-registration course, through the training grades, to life as a senior independent practitioner. Although this article concentrates on undergraduate teaching, many of the principles apply equally to interprofessional learning by postgraduates.

An example of interprofessional education: The New Generation Project

At Southampton, we collaborate with the University of Portsmouth in providing interprofessional education to approximately 1500 students per year [5]. This activity is called the New Generation Project and has been in place since 1999.

All healthcare students at the Universities of Southampton and Portsmouth are involved. The disciplines are: audiology, medicine, midwifery, nursing, occupational therapy, paramedics, pharmacy, physiotherapy, podiatry, radiography, and social work. It comprises 8 weeks of full-time study divided into three units (Table 1). In each unit, the students are allocated to multidisciplinary groups of 8 to 10 students each (Figure 1). A facilitator is assigned to each group.

The theoretical frameworks underpinning the units are those of social and experiential learning [9]; there is evidence that experiential learning in particular is valued by students in this context [10]. The learning is structured around collaboration and reflection. The first unit occurs on campus, but the other two take place in the practice setting where the students are based at the time; this draws on the concept of situated learning, and the "authenticity of practice" maximises the relevance of the learning activities [2, 4, 11].

Table 1. The units of study in the New Generation Project.

Unit 1: 1st year, 2 weeks.	Students reflect on the nature of teamwork, consider a clinical scenario, and research the role of different professions in the care of the patient.
Unit 2: 2nd year, 2 weeks.	Students perform an audit into an aspect of patient care or public health.
Unit 3: Final year, 4 weeks.	Students identify an area of service need and develop a case for change and its implementation.

Figure 1. A New Generation Project group at Southampton

Participation is compulsory for all students (unlike the elective courses at other institutions [3]). All three units are summatively assessed, and all of them must be passed if the student is to progress to the next phase of the course. Techniques include group presentations and reflective accounts. We believe assessment is vital to demonstrate the value placed by faculty on the aims of interprofessional education.

In our experience, the quality of facilitation is a key component in making interprofessional learning a success. As others have noted [10], modelling of appropriate behaviours and attitudes by the facilitator is essential. Faculty development may be required to allow facilitators to work through their own prejudices and social biases [6, 12]. Facilitators need to be able to cope with dysfunctional groups; disagreements and difficulties can be learning opportunities if properly facilitated [2].

The unit structure of the New Generation Project is a two-edged sword. From an administrative point of view, it is convenient. It also allows students to concentrate on the learning activities without competing distractions from other curricular events. However, its temporal separation from other components of the course may promote the view among students and staff that it is an "optional extra" rather than a vital, integrated part of the overall learning experience. Ideally, interprofessional education should run as a continuous theme throughout the course, seamlessly linked to all other components [6].

Finally, evaluation of the course is considered essential. Not only is it the key to quality improvement, it is also the way to demonstrate the value of interprofessional learning to funding bodies and to potentially sceptical colleagues.

Other models of interprofessional education

There is great diversity in the pedagogy of interprofessional education as reported in the literature [3, 6, 11, 13]. Common techniques include problem-based learning, experiential learning and simulations. They may occur in the classroom, the clinical skills laboratory or clinical placements.

Student training wards have been notably successful [13-15]. Authentic patients are admitted to training wards run by health care students, typically in their final year of training. The team of students is responsible for patient care and management under the guidance and supervision

of qualified professionals. Interestingly, there is evidence that patients are more satisfied with their care on training wards than non-training wards [15].

Barriers to successful implementation

The introduction of interprofessional learning needs to overcome significant barriers [3, 6]. The first is resistance from staff. Some qualified healthcare professionals have negative attitudes towards interprofessional education and express scepticism about its value [2, 3, 12]. Not only does this cynicism, which can be seen in all healthcare disciplines, translate into resistance towards interprofessional education, it can also influence students as a powerful part of the "hidden" or "informal" curriculum [16]. As discussed later in this article, an important way of countering this attitude is to provide good evidence that interprofessional education is of value, and so more well-designed research is required [4, 6, 17]. Until this research is published, there will be continuing criticism from some quarters that interprofessional education lacks an evidence base to support it. This criticism may be the result of unjustified prejudice, since there are many other areas in medical education in which the evidence is somewhat flimsy, and interprofessional education has at least as much evidence to support it as some more widely adopted educational methods.

Second, resources need to be considered. Recruiting sufficient facilitators and finding enough physical space for the learning activities can be problematic. Our experience with 1500 students a year shows that finding enough rooms for the groups of students to use is never straightforward. Another required resource is appropriate funding – the different universities, faculties and schools need to agree how funding is to be sourced and allocated [3].

Third, space must be found in the timetable [6]. When interprofessional learning was introduced at Southampton, the curricula were already full. Therefore, implementation required significant changes to the existing curricula. These changes can meet with significant resistance from sceptical faculty.

Fourth, there is the issue of social status within the groups of learners [3]. Gender, class and professional identity may combine to create power dynamics within the group. These dynamics are based largely on the stereotypes that interprofessional education is designed to address. Therefore, good facilitation can use this as a positive learning experience, but with poor facilitation there is a risk that negative attitudes and working practices could become entrenched.

What is the evidence?

Although the benefits of interprofessional education may seem obvious to its proponents, we should consider the evidence that it delivers the desired learning outcomes. Unfortunately, much of the literature on interprofessional education is descriptive and anecdotal [9, 17] and there is a need for more rigorous research in this area [4, 6]. Nevertheless, there is an increasing body of evidence that learners value interprofessional education and appreciate its benefits [4, 10, 18-20].

There is also evidence that attitudinal change can occur – negative stereotyping of other professionals can be reduced [14]. However, Barnes et al described a program of interprofessional education in which there was no change in negative attitudes to other professions as a result of participation [21]. Analysing this finding in terms of All port's contact hypothesis, the authors concluded that possible causes were insufficient opportunities for successful joint work in small groups, a lack of exploration the differences as well as the similarities between professional groups, and a perception that the other participants were not typical of their profession as a whole [21, 22]. The last was possible because the participants chose to take the course; thus a positive experience of another individual may not be extrapolated to the profession as a whole (e.g. "only nice doctors take this course"). If participation is compulsory there is less scope for seeing other participants as atypical. Another feature of the contact hypothesis is that positive prior expectations by the group members are important in delivering desired changes in attitudes [22]. At Southampton, many students have negative prior expectations of interprofessional learning, which could inhibit a re-evaluation of their prejudices in this context. A negative stance from faculty can reinforce these attitudes [2, 3, 12].

Our experience at Southampton is that student evaluations of interprofessional learning modules are generally positive, although we often find that evaluations performed several months after a module are lower than those per-

formed immediately at the end of the module (Lueddeke G 2009, personal communication); the significance of this phenomenon is unclear.

Improving quality

There are a number of ways in which we could help interprofessional education to grow. I would like to highlight four:
• research,
• theory,
• student leadership,
• assessment.

The need for more research has been mentioned previously. Good research will point to ways in which our educational interventions can be improved. Furthermore, the best ammunition that a champion of interprofessional education can use in justification is rigorous evidence.

There are a number of ways in which educational theory can guide the development of teaching and learning [9]. It can help clarify concepts, specify learning objectives, suggest appropriate roles for learners and faculty, and provide a framework for measuring impacts and outcomes. However, the interprofessional education literature does not often address underlying theory, and there is a need for workers in this field to be more aware of the theoretical basis of their work [9, 17, 22].

In general, it is rare for undergraduates to be involved in the design of the courses they study. However, there is evidence that undergraduates are able to engage successfully in curriculum development [23]. Relinquishing power to students in this way may go against the instincts of many (perhaps most) teachers, but there is no reason why intelligent and motivated adult learners should not be able to develop a course, especially if they have good educational guidance from faculty. It seems likely that students would be more highly motivated in a course they had developed themselves, and this might be a way of reducing the level of scepticism with which some students approach interprofessional education. It might also be that such a course would address the needs of students more directly. Properly researched and evaluated, student leadership could provide significant educational benefits.

As mentioned previously, at Southampton we believe appropriate assessment of the students is essential. However, a systematic review found that only a minority of interventions in this area were summatively assessed [17]. On the principle that assessment drives learning [24], assessments and desired outcomes should be properly aligned. Without such assessment, it may be difficult to convince students that the outcomes are important, and the desired learning may not occur.

Conclusion

The aim of interprofessional learning is to improve the performance of multidisciplinary healthcare teams. The growing literature on the subject describes a wide variety of exciting and innovative techniques, and there is increasing attention to the educational theory underpinning them. Although more research is required, there is evidence of the benefits of this type of learning. Our experience at Southampton is that it is well worth rising to the challenge of implementing interprofessional education.

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