

CHAPTER 1

INTRODUCTION

1.1 WARFARIN EMBRYOPATHY

Coumarin derivatives are anticoagulants that cross the placenta. Warfarin is one such derivative, and when taken in pregnancy, produces teratogenic effects in the fetus known as warfarin embryopathy (WE) or the fetal warfarin syndrome. Warfarin also causes fetal central nervous system (CNS) abnormalities, spontaneous abortion, and stillbirth (Schardein, 2000; Gorlin, Cohen and Hennekam, 2001). Previously, warfarin was the oral anticoagulant prescribed for pregnant women for the treatment and prevention of thrombophlebitis, pulmonary embolic disease, hypertension, pre-eclampsia, coagulation disorders, and for anticoagulation of prosthetic heart valves (Hall, Pauli and Wilson, 1980; Schardein, 2000). The latter indication is the commonest reason for pregnant women in South Africa (SA) to be on warfarin since rheumatic heart disease is still prevalent and often results in heart valve replacement.

The most consistent feature of WE is nasal hypoplasia due to underdevelopment of the nasal cartilage, and choanal atresia may also occur (Hall et al., 1980; Schardein, 2000; Gorlin et al., 2001; Van Driel, Wesseling, and Sauer, et al., 2003). The small nares (with/without choanal atresia) produce respiratory distress in up to 70% of these babies in the neonatal period, and while these symptoms improve as the baby grows, the nose does not appear to show complete catch-up growth in later years (Gorlin et al., 2001). Warfarin embryopathy encompasses other skeletal abnormalities. Stippling of the epiphyses is common, with the lumbosacral

vertebrae, calcanei and proximal femoral epiphyses mainly being involved. The stippling usually disappears by the first year of life, and though short stature may result in about 35% of patients, asymmetric bone growth does not appear to occur (Hall et al., 1980, Gorlin et al., 2001). Other skeletal defects include hypoplasia of the extremities (from rhizomelic limb shortening to mild brachydactyly), dystrophic nails, and abnormal skull development (Gorlin et al., 2001)

The fetal CNS sequelae of maternal warfarin ingestion in pregnancy include fetal cerebral haemorrhage in the second and third trimesters and around the time of labour, and structural abnormalities. Porencephalic cysts may result from the cerebral haemorrhage. The structural CNS abnormalities noted include hydrocephalus, dorsal midline dysplasia of the brain (resulting in agenesis of the corpus callosum, Dandy–Walker malformations, encephalocoeles, and midline cerebellar atrophy), and ventral midline dysplasia (resulting in optic atrophy). Complications of these malformations include seizures, mental retardation, spasticity, deafness, and blindness (Hall et al., 1980; Van Driel et al., 2003).

1.1.1 Pathogenesis of warfarin embryopathy and CNS abnormalities

The anticoagulant effects of warfarin appear to be due to the unbound fraction of the drug, and it is this fraction that crosses the placenta. It would appear that fetuses are at risk for exposure to higher concentrations of this unbound fraction (and its teratogenic effects) for the following reasons:

- A greater unbound fraction of warfarin has been found in the serum of pregnant women than non-pregnant women, which then crosses the placenta.
- Fetuses have relatively high concentrations of bilirubin that displace the unbound fraction of warfarin from albumin into serum.

- The hepatic glucuronide pathway, necessary to produce water soluble warfarin metabolites for renal elimination, is immature in the fetus, thus reducing fetal elimination of warfarin. (Bajoria, Sooranna and Contractor, 1996).

The pathogenesis of WE and the CNS abnormalities after fetal warfarin exposure is not clear.

The following hypotheses have been proposed:

Hypothesis 1

Reduced vitamin-K is required for the post-translational carboxylation of many proteins so that gamma-carboxyglutamyl residues can be formed which can bind calcium. Such proteins include clotting factors and osteocalcins in bone (Hall et al., 1980; Pauli, Lian, and Mosher et al., 1987). Coumarins inhibit vitamin-K reductase, decreasing the amount of active vitamin-K, and thereby decreasing the post-translational carboxylation of these proteins. It would appear that osteocalcins are present in humans by 6 to 8 weeks gestation (Pauli et al., 1987). If inhibition of post-translational carboxylation of osteocalcins (and consequent reduction in calcium binding) occurs at this critical time in fetal ossification, it may explain the skeletal abnormalities of WE (Hall et al., 1980; Pauli et al., 1987; Menger, Lin, and Toriello et al., 1997).

Hypothesis 2

It has been hypothesised that the CNS malformations seen in fetuses exposed to warfarin may represent deformations rather than malformations. Warkany (1976) proposed that fetal cerebral haemorrhage occurring after organogenesis of the CNS was complete, may result in scarring and subsequent dysharmonic growth of the CNS, and therefore produce the abnormalities noted (Hall et al., 1980).

Hypothesis 3

Recently, it has been shown that warfarin inhibits the activity of the product of the *arylsulfatase E (ARSE)* gene, located on chromosome Xp22.3 (Franco, Meroni, and Parenti et al., 1995). This gene is mutated in patients with X-linked recessive chondrodysplasia punctata (CDPX), which has similar clinical features to WE. Although the exact mechanism of how ARSE deficiency causes skeletal abnormalities is not known, sulfatases are known to be important in cartilage and bone development. It therefore seems reasonable to hypothesise that disruption of the pathway in which the product of the *ARSE* gene is involved in the fetus, either by genetic mutation (as in CPDX), or by pharmacological means (such as warfarin), may explain the deformities seen in CPDX and the WE (Franco et al., 1995). At present it is not known whether warfarin's interaction with other genes also contributes to the pathogenesis of WE and CNS abnormalities of warfarin-exposed fetuses (see section 1.2.5 for further discussion).

1.2 LITERATURE REVIEW

1.2.1 Incidence of warfarin embryopathy and poor pregnancy outcomes

In the first extensive literature review on WE, Hall, Pauli and Wilson (1980) showed that ingestion of warfarin in the first trimester, particularly between six and nine weeks, produced features of the syndrome. Ingestion of warfarin at any time in pregnancy (including the second and third trimesters) resulted in CNS malformations. The authors estimated that, at best, two thirds of babies born to mothers taking warfarin would be normal, 1/6 of fetuses exposed to warfarin were aborted or stillborn, and a further 1/6 were abnormal. Of those born with CNS malformations, most did poorly, while 50% with the skeletal abnormalities of WE did well.

Considering local data, a study conducted at Baragwanath Hospital in 1989 prospectively followed 50 pregnancies in 49 patients with prosthetic heart valves (Sareli, England, and Berk et al., 1989). All patients received warfarin during the first and second trimesters, and 11 patients also received dipyridamole. Overall, the poor outcome rate for these pregnancies was 40%, with 4% of newborns noted to have WE. There were no maternal deaths or thromboembolic complications (TEC) associated with pregnancy. Another review of pregnancy outcomes in a group of SA women from the Western Cape with artificial heart valves was published in 2001 (Hall, Olivier, and Rossouw et al., 2001). Sixty-eight percent of women (24/49) received warfarin during the first trimester, and 28 % of these women experienced pregnancy loss. Of the liveborn babies, 3/49 (6%) were noted to have features consistent with WE. Three women died in the post-partum period; one due to accidental head injury and intracranial bleed while on heparin, and the other two due to TEC.

A study was published in 2000, reviewing outcomes of pregnant patients with heart valve replacements in New Zealand from 1972 to 1992 (Sadler, McCowan, and White et al., 2000). The anticoagulation regimen for pregnant women with mechanical prosthetic valves during the mid 1980's in that country was to switch patients from warfarin to heparin at the time the pregnancy was diagnosed, and only restart warfarin after delivery. Not all patients followed the regimen, and the authors reported that pregnancy loss was 70% (19/25) in women who took warfarin throughout pregnancy. No TEC occurred in women taking warfarin throughout pregnancy, but 4/14 women who were on heparin during their pregnancies suffered this complication. One of these four women died as a result of a coronary artery embolus. This study reports one of the highest poor outcome rates in women taking warfarin in pregnancy, but the study numbers were small.

Chan, Anand and Ginsberg (2000) published a systematic review of the literature regarding the fetal and maternal risks of anticoagulation in pregnant women with mechanical heart valves. They showed that WE occurred in 6.4% (35/549) of infants whose mothers took warfarin throughout pregnancy, and in this same group, the spontaneous miscarriage rate was 24.7% (196/792). Thromboembolic complications occurred in 31/788 (3.9%) of these women, and 10/561 (1.8%) maternal deaths were reported. When heparin replaced warfarin at or before six weeks of pregnancy, no embryopathy was noted, but the risk of maternal TEC increased to 9.2% (21/229). In this latter group, 7/167 (4.2%) mothers died.

A more recent Italian retrospective analysis of the pregnancy outcomes in women with prosthetic heart valves taking warfarin, raised the question of whether warfarin dose was important. The study found a poor outcome (spontaneous abortion, stillbirth or congenital defect) in 42% (30/71) of the pregnancies of the study subjects (Cotrufo, De Feo, and De Santo, et al., 2002). The authors emphasized that poor outcome was significantly related to warfarin dose: 90% of women taking ≥ 5 mg warfarin daily had a poor fetal outcome. No maternal deaths or TEC occurred in the study group. Again, study numbers were small (71 pregnancies in 52 patients), and the importance of the relationship of warfarin dose to poor pregnancy outcome still needs to be verified by other, larger, studies.

1.2.2 Effects of other anticoagulants

Women with prosthetic heart valves are at high risk for TEC during the hypercoagulable state of pregnancy. Their anticoagulation during pregnancy therefore has to be carefully considered. Warfarin is effective in preventing thrombosis, but it crosses the placenta and is teratogenic. Alternative anticoagulants, like heparin, do not cross the placenta, but are not as effective as warfarin in preventing clotting of artificial valves. A review by Ginsberg, Greer

and Hirsh (2001), showed that the risks of maternal TEC in women with prosthetic heart valves are higher when heparin rather than warfarin is used in the first trimester or throughout pregnancy (9.2% (21/229) and 33% (7/21) respectively). The number of maternal deaths (from all causes) in these two groups was 7/167 (4.2%) and 3/20 (15%) respectively. They also reported that the fetal risks for malformation are lower in these categories than when warfarin is used. No instances of WE were noted if heparin was used throughout pregnancy. Within the group of women using heparin in the first trimester, 0/108 of those who were changed from warfarin to heparin at or before six weeks had babies with WE, whereas 4/36 (11%) of those switched after six weeks had babies with WE. These latter figures emphasise that six weeks appears to be a critical period in the causation of WE.

Other serious side effects of heparin when used long term include osteoporosis and thrombocytopenia (Ginsberg et al., 2001; Cotrufo et al., 2002). Heparin also causes spontaneous abortion, stillbirth and premature delivery in proportions similar to those seen in women taking warfarin during pregnancy (Hall et al., 1980). Hall et al. (1980) reported that of fetuses exposed to heparin, 1/8 were stillborn, 1/5 were born prematurely, and, at best, a normal outcome could be expected in two thirds of fetuses exposed to heparin in pregnancy. Similarly, Ginsberg et al. (2001) showed that 42.9 % (9/21) of pregnancies resulted in fetal wastage when heparin was used continuously in pregnancy, compared to 33.6 % (266/792) when warfarin was used continuously.

1.2.3 Current recommendations for anticoagulation in pregnancy

No clinical trials have been conducted to provide guidelines for optimal antithrombotic therapy in pregnant patients, especially those with prosthetic heart valves (Chan et al., 2000;

Sadler et al., 2000; Ginsberg et al., 2001; Cotrufo et al., 2002). Current recommendations are that unfractionated heparin or low-molecular weight heparin should be used in pregnant women for the prevention and treatment of venous thromboembolism and prophylaxis of venous thromboembolism in women with a known thrombophilia (like antithrombin III deficiency). Prevention of pregnancy loss in women with antiphospholipid antibodies can be achieved with low-dose aspirin and heparin (Ginsberg et al., 2001).

The American Heart Association recommends that warfarin should be strongly considered as part of the anticoagulation regimen in women with prosthetic heart valves because of the high risk of TEC (Chan et al., 2000; Ginsberg et al., 2001; Hirsh, Puster and Ansell, 2003).

If warfarin is used, the international normalized ratio (INR) should be maintained between 2.0 and 3.0 for patients with bioprosthetic valves or bi-leaflet valves, and between 2.5 and 3.5 for patients with most other mechanical valves (Hirsh et al., 2003). Women who refuse warfarin therapy in pregnancy need to understand the risks of the alternative therapy (namely high-dose intravenous heparin therapy throughout pregnancy), and that low-molecular weight heparin is not approved for use in any patients with mechanical prosthetic heart valves. (Hirsh et al., 2003).

The following anticoagulation regimens are therefore available to pregnant women with prosthetic heart valves:

1. Heparin is used throughout the pregnancy (and possibly even from before conception).
2. Warfarin is used throughout the pregnancy, changing to heparin at about 38 weeks gestation with a planned induction of labour.

3. Heparin is used during the first trimester of pregnancy (particularly between weeks six and nine), switching back to warfarin from the second trimester to 37 completed weeks of pregnancy, and then switching to heparin until after the planned delivery.

Regimen 3 is an attempt to balance the risk of fetal warfarin effects and maternal risk of TEC and death (Chan et al., 2000; Sadler et al., 2000; Ginsberg et al., 2001).

Regimens combining warfarin and heparin are problematic in that:

- Patients usually require hospital admission for the entire duration of heparin administration.
- Frequent monitoring of serum markers of anticoagulation is required during both warfarin and heparin therapy if anticoagulation is to be effective in pregnancy.
- Women on warfarin often present after the first trimester and therefore too late to prevent the teratogenic effects of the drug.
- Women presenting in the first trimester often present after 6 weeks, which also appears to be suboptimal in terms of preventing a poor pregnancy outcome.
- The risks to the fetus are not insignificant even if only exposed to warfarin in the second and third trimester.
- Regimen 3 is by no means followed by all clinicians. Many believe that the thromboembolic risks to women with prosthetic heart valves are too high to warrant stopping warfarin at any time during pregnancy.
- The spontaneous abortion rate appears to be similar, or higher, when heparin is used in the first trimester.

1.2.4 Warfarin dosage in pregnancy

As already stated, pregnancy is a hypercoagulable state. This is the result of increases in coagulation factors II, VII and X, decreases in the natural anticoagulant protein S, and inhibition of fibrinolysis (Brooks, Rutherford, and Gould, et al., 2002). These effects remain until about six weeks post-partum, and theoretically make pregnant women more resistant to anticoagulation with drugs like warfarin. Indeed, Brooks et al (2002) showed that women in the immediate post-partum period required significantly higher doses of warfarin to achieve INR results in the recommended range of 2 – 3 than a control group of non-pregnant women taking warfarin. If pregnant women generally do require higher doses of warfarin to maintain adequate anticoagulation, this may further increase the teratogenic levels of warfarin to which their fetuses are exposed, though this supposition has not been verified in clinical studies.

1.2.5 Patient understanding of the risks of warfarin therapy

Studies have been conducted assessing patient knowledge and perceptions of their warfarin therapy. These have mainly concentrated on patients >60 years of age taking warfarin for either atrial fibrillation or venous thromboembolic disease, and not specifically on women of childbearing age (Lip, Kamath, and Jafri, et al., 2002; Nadar, Begum, and Kaur, et al., 2003). These surveys show that patients have a very limited knowledge of the underlying medical condition requiring them to be on warfarin, and the consequences of warfarin therapy. Further, the one study highlighted that patients from different ethnic groups had different levels of knowledge regarding the risks, actions, and benefits of warfarin and atrial fibrillation (Lip et al., 2002). Where women of childbearing age were included in the studies, their perceptions about the impact of warfarin on pregnancy were not specifically examined (Nadar et al., 2003). No studies could be found assessing patients' knowledge of the effects of warfarin in pregnancy.

1.2.6 Future considerations

Given that anticoagulation therapies like heparin are suboptimal in women with prosthetic heart valves during pregnancy, it would appear that warfarin will remain part of their anticoagulation regimen for the foreseeable future. Other strategies to improve the pregnancy outcomes in these women in the face of warfarin therapy therefore need to be found. Genetic studies may provide some answers if women can be identified who are at high risk for having poor pregnancy outcomes when they take warfarin.

Genetic variants have been found in the CYP2C9 hepatic microsomal enzyme that primarily inactivates warfarin (Aithal, Day, and Kesteven, et al., 1999; Higashi, Veenstra, and Kondo, et al., 2002). Individuals with polymorphisms of the *CYP2C9* gene described to date, different to the wild-type genotype, metabolise warfarin less efficiently and are therefore at higher risk for overanticoagulation and bleeding on standard doses of warfarin. The *CYP2C9*1*, *CYP2C9*2*, and *CYP2C9*3* alleles are the commonest variants in individuals of European descent (Higashi et al., 2002). *In vitro*, these variants metabolise warfarin with 100%, 12% and less than 5% efficiency, respectively (Aithal et al., 1999). The African American population has low frequencies of the *CYP2C9*2* and *CYP2C9*3* variants, but a unique variant, *CYP2C9*5*, was found to occur in 3% of this population (Higashi et al., 2002). The *CYP2C9*5* allele has 8 – 18% *in vitro* clearance of warfarin as compared to the wild-type (*CYP2C9*1*) allele (Dickmann, Rettie, and Kneller, et al., 2001).

Women homozygous for the wild-type *CYP2C9*1* allele, who require the highest maintenance doses of warfarin, may be at most risk for having poor pregnancy outcomes or fetuses adversely affected by warfarin. Alternately, women who are heterozygous or homozygous for the *CYP2C9*2*, *CYP2C9*3* and *CYP2C9*5* alleles and metabolize warfarin

more slowly, may have lower warfarin requirements, and may be at high risk if given normal warfarin doses. Similarly, fetuses whose genotype causes them to metabolise warfarin more slowly than those with the wild-type *CYP2C9* allele, may be at highest risk for the adverse effects of warfarin. An interaction between the maternal and fetal genotype may also be anticipated. For instance, if a mother who is homozygous for the wild-type allele (and on a high warfarin dose) carries a fetus who is hetero- or homozygous for an allele with diminished warfarin metabolizing properties, that fetus may be exposed to particularly high levels of warfarin.

At present the frequencies of the *CYP2C9* alleles in the black SA population are not known. With this knowledge, it may be possible to correlate the genotype of the parents and/or fetus and the pregnancy outcome and incidence of fetal abnormalities. Should this correlation be possible, it would allow for more accurate counselling of women who take warfarin about their risks of having a poor pregnancy outcome or affected baby. In addition, knowledge of a woman's *CYP2C9* genotype may allow clinicians to appropriately adjust her warfarin dose and minimize haemorrhagic complications.

Coagulometers allowing for point-of-care (POC) monitoring of INR have been available since 1987, and therefore allow for patient self-testing at home (Hirsh et al., 2003). Further, POC self-testing has allowed for self-management of warfarin dose by patients. Home testing of INR for patients on warfarin with relevant dose adjustment is therefore similar to blood glucose monitoring and insulin dose adjustment in diabetic patients. Although some researchers have shown a reduction in the rate of haemorrhagic and thromboembolic complications between self-testing and usually managed patients (Lafata, Martin, and Kaatz et al., 2000; Horstkotte and Piper, 2004), other groups have reported no long term difference

in the INR falling in the therapeutic range between self-managed and anticoagulation clinic-managed patients (Gadisseur, Kaptein, and Breukink-Engbers et al., 2004). Point-of-care self-testing and self-management offers flexibility of lifestyle to patients on warfarin, and appears to be no worse than usual care provided by anticoagulation clinics (Gadisseur, Breukink-Engbers, and van der Meer et al., 2003; Gadisseur et al., 2004). Lafata et al (2000) showed that home INR testing and patient self-management was the most cost-effective way to manage patients on warfarin when compared to management in a usual outpatient setting or specialized anticoagulation clinic. It seems feasible to consider that POC testing and patient self-management may benefit patients even in a developing country like SA, and result in a cost saving for these patients who require such intensive medical follow-up. These advantages may apply particularly to those patients requiring warfarin who do not have easy or regular access to medical care, either because of geographical or financial constraints.

1.3 STUDY OBJECTIVES AND AIMS

About 40 to 45 pregnant women taking warfarin are followed at the Obstetric Cardiac Clinic at the Chris Hani-Baragwanath (CHB) Hospital annually (Dr Norma Pirani, Senior Obstetrician, CHB, personal communication). This clinic represents but one such centre in SA. Because rheumatic heart disease necessitating heart valve replacement is still common in SA, hundreds of women with prosthetic valves nationwide are annually exposed to warfarin during their pregnancies. In addition, warfarin is often prescribed for women with critical mitral stenosis when they are pregnant, and many clinicians still use warfarin to treat venous thromboembolism in pregnancy. The number of fetuses affected with WE and women with poor pregnancy outcomes as a result of warfarin therefore represents a significant public health issue. Before policies can be established regarding the care of affected liveborns and

the prevention of poor outcomes in this group of women, accurate data are required about the extent of this problem in SA.

It is our clinical impression, from the patients seen at the Genetic Counselling Clinics of the Department of Human Genetics, National Health Laboratory Service and University of the Witwatersrand, that women in the local population on warfarin have poor obstetric histories. Further, the management practices with regard to anticoagulation in pregnancy are by no means standardised, and many clinicians believe that the adverse effects of warfarin in pregnancy are overemphasised. The study was therefore conducted to clarify the situation at the CHB Hospital, Johannesburg, and the aims and objectives can be summarized as follows:

- To describe the pregnancy outcomes of black SA women of childbearing age on warfarin therapy in the specified region.
- To assess the level of awareness of the effects of warfarin in pregnancy in the study population, in a SA urban setting.
- To determine management practices, as reported by patients in the study population, with particular emphasis on referral for genetic counselling and anticoagulation management in pregnancy.

1.4 LIMITATIONS OF THE STUDY

Although the study data were collected prospectively, the information sought was of a retrospective nature, and required patients to recall events that may have occurred many years previously. Some of the information may therefore not be entirely accurate, particularly as regards recall of exact timing of changes in medication and exact timing of events during past pregnancies. Medication used during the pregnancies was not verified by examining old bedletters.

Patient records in the Department of Human Genetics, National Health Laboratory Service and University of the Witwatersrand, were checked to see if any of the children reported as being abnormal at birth had been seen at any of our Genetic Clinics. As far as we are aware, none of these children who are still alive was brought to any of our genetic counselling clinics, despite an invitation in all cases to do so. The accuracy of their abnormalities, as reported by their mothers, has therefore not been verified.

The study cohort represents an urban population serviced by a tertiary level referral hospital. Results obtained can therefore not be extrapolated to rural communities serviced by regional or secondary tier hospitals, or indeed, to other urban regions where levels of service delivery may differ.

1.5 UNIQUENESS OF THE STUDY

This study examines pregnancy outcomes and management practices as regards anticoagulation in pregnancy and referral for genetic counselling in women taking warfarin in pregnancy from the patient's point of view. To our knowledge, the level of awareness in a group of childbearing-age women taking warfarin regarding the effects of their medication has not been previously evaluated, making this study unique.

The study cohort is also unique in that it is the largest in SA to be investigated regarding pregnancy outcomes in women taking warfarin.